



Components, Mechanisms of Action, Success Under Greenhouse and Field Condition, Market Availability, Formulation and Inoculants Development on Biofertilizer



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Abstract

A bio-fertilizer is a modernized form of organic fertilizer into which beneficial microorganisms have been incorporated. This review mainly focus on components, mechanisms, market availability on Biofertilizer. Nitrogen fixers, potassium solubilizers, phosphorus solubilizer and phosphorus mobilizers that are applied exclusively or in combination with fungi are the components of Biofertilizer. Various types of microbial cultures and inoculants are available on the market today and these have rapidly increased because of the advances in technology. There is a success to improve mineral uptake, release minerals from soil and organic matter, enhance plant hormone production, induce systematic resistance mechanisms, and induced root systems under greenhouse and field condition.

Abbreviations: EIAR: Institute of Agricultural Research; NABRC: National Agricultural Biotechnology Research Center; CMC: Cattle manure compost; ACC: aminocyclopropane-1-carboxylate; IAA: Indol acetic acid; PSB: phosphate-solubilizing bacteria; BNF: biological N₂ fixation; N: Nitrogen; VAM: Vesicular Arbuscular Mycorrhiza

Introduction

The utilization of synthetic fertilizers is a reason for air and ground water pollution as a result of eutrophication of water bodies [1]. As reported by [2], the practice of using chemical fertilizers and pesticides accelerates soil acidification, weakens the roots of plants as a result plants be susceptible to different diseases. The Negative effects posed to soil by the application of chemical fertilizers obligated human being to search for an alternative fertilizer the so-called biofertilizer to increase soil fertility and crop production in sustainable farming. These biofertilisers are essentially the microbial inoculants of living cells which aid in nutrient assimilation through the process of colonization, mobilization or solubilisation of nutrients. These biofertilisers that contain beneficial microorganisms accelerate and improve plant growth and protect plants from pests and diseases [3]. Biofertilisers would have the key role in productivity and sustainability of soil and also protect the environment as eco-friendly and cost-effective inputs for the farmers [4].

The commercial history of bio-fertilizer began with the launch of "Nitragin" by Nobbe and Hilther in 1895. This was followed by the discovery of Azotobacter and then Blue-green algae and a host of other microorganisms which are being used till date as bio-fertilizer [5]. Biofertilizers keep the soil environment rich in all kinds of macro and micro nutrients via nitrogen fixation, phosphate and potassium solubilisation or mineralization, release of plant growth regulating substances, production of antibiotics and biodegradation of organic matter in the soil [6].

Components of Biofertilizer

A bio-fertilizer is a modernized form of organic fertilizer into which beneficial microorganisms have been incorporated. In a broad sense, the term bio-fertilizer may referred as all organic resources that utilized for plant growth that are rendered in available form for plant absorption through microorganisms or plant associations or interactions [4]. The bio-fertilizers components include: nitrogen fixers, potassium solubilizers, phosphorus solubilizer

and phosphorus mobilizers, that are applied exclusively or in combination with fungi. Most of the bacteria used in bio-fertilizers have close relationship with plant roots. Rhizobacterium has symbiotic interaction with legume roots, and Rhizobacteria inhabit root surfaces or rhizosphere soil [4]. The phosphorus microorganisms mainly bacteria and fungi make insoluble phosphorus available to the plants.

Several soil bacteria and few species of fungi possess the ability to convert insoluble phosphate in soil into soluble forms by secreting organic acids. These acids lower the soil pH and bring about the dissolution of bound forms of phosphate. While Rhizobium, blue-green algae, and Azolla are crop specific, bioinoculants such as Azotobacter, Azospirillum, phosphorus solubilizing bacteria (PSB), and Vesicular Arbuscular Mycorrhiza (VAM) could be regarded as broad-spectrum bio-fertilizers. VAM are fungi that are found associated with majority of agricultural crops and enhanced accumulation of plant nutrients. Reports show that VAM stimulate plant by physiological effects or by minimizing the severity of diseases caused by soil pathogens. Examples of free-living nitrogen fixing bacteria are obligate anaerobes *Clostridium pasteurianum* obligate aerobes, facultative anaerobes, photosynthetic bacteria *Rhodospirillum rubrum*, cyanobacteria (*Azotobacter*), and some Methanogens. The most commonly used potassium solubilizer is *Bacillus mucilaginosus*, while phosphorus solubilizers are *Bacillus megaterium*, *Bacillus circulans*, *Bacillus subtilis* and *Pseudomonas striata*.

Mechanisms of Plant Growth Promotion

According to [7], PGPR mediated plant growth promotion occurs by the alteration of the whole microbial community in rhizosphere niche through the production of various substances. Generally, PGPR promote plant growth directly by either facilitating resource acquisition (nitrogen, phosphorus and essential minerals) or modulating plant hormone levels, or indirectly by decreasing the inhibitory effects of various pathogens on plant growth and development in the forms of biocontrol agents [8].

Direct Mechanisms

Nitrogen Fixation: Nitrogen (N) is the most vital nutrient for plant growth and productivity. Since the plants have no ability to utilize the atmospheric N_2 , it converted into plant-utilizable forms by biological N_2 fixation (BNF) which changes nitrogen to ammonia by nitrogen fixing microorganisms using a complex enzyme system known as nitrogenase [9]. Biological nitrogen fixation occurs, generally at mild temperatures, by nitrogen fixing microorganisms, which are widely distributed in nature [10]. Furthermore, BNF represents an economically beneficial and environmentally sound alternative to chemical fertilizers [11]. Nitrogen fixing organisms are generally categorized as (a) symbiotic N_2 fixing bacteria including members of the family rhizobiaceae which forms symbiosis with leguminous plants (e.g. rhizobia) [12,13] and non-leguminous trees (e.g. *Frankia*) and (b) non-symbiotic (free living, associative and endophytes) nitrogen fixing forms such as cyanobacteria (*Anabaena*, *Nostoc*), *Azospirillum*, *Azotobacter*, *Gluconacetobacter diazotrophicus* and *Azococcus* etc [14].

However, non-symbiotic nitrogen fixing bacteria provide only a small amount of the fixed nitrogen that the bacterially-associated host plant requires [8]. Symbiotic nitrogen fixing rhizobia within the rhizobiaceae family (α -proteobacteria) infect and establish symbiotic relationship with the roots of leguminous plants. The establishment of the symbiosis involves a complex interplay between host and symbiont [15] resulting in the formation of the nodules wherein the rhizobia colonize as intracellular symbionts. Plant growth-promoting rhizobacteria that fix N_2 in non-leguminous plants are also called as diazotrophs capable of forming a non-obligate interaction with the host plants [8]. The process of N_2 fixation is carried out by a complex enzyme, the nitrogenase complex [9]. Most biological nitrogen fixation is carried out by the activity of the molybdenum nitrogenase, which is found in all diazotrophs [16].

Phosphate Solubilization: Majority of Phosphorus (P) in the soil are insoluble forms and this causes shortage of the availability of P because plants absorb it only in two soluble forms, the monobasic ($H_2PO_4^-$) and the dibasic (HPO_4^{2-}) ions [14]. 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 [17]. But regular application of phosphate fertilizers is not only costly but is also environmentally undesirable. This has led to search for rhizosphere, phosphate-solubilizing bacteria (PSB) that are considered as promising biofertilizers since they can supply plants with P from sources otherwise poorly available by various mechanisms [18]. bacterial genera like *Azotobacter*, *Bacillus*, *Beijerinckia*, *Burkholderia*, *Enterobacter*, *Erwinia*, *Flavobacterium*, *Microbacterium*, *Pseudomonas*, *Rhizobium* and *Serratia* are reported as the most significant phosphate solubilizing bacteria [14].

Typically, the solubilization of inorganic phosphorus occurs as a consequence of the action of low molecular weight organic acids which are synthesized by various soil bacteria [18]. Conversely, the mineralization of organic phosphorus occurs through the synthesis of a variety of different phosphatases, catalyzing the hydrolysis of phosphoric esters [8]. Importantly, phosphate solubilization and mineralization can coexist in the same bacterial strain. Besides providing P to the plants, the phosphate solubilizing bacteria also augment the growth of plants by stimulating the efficiency of BNF, enhancing the availability of other trace elements by synthesizing important plant growth promoting substances [19,18].

Siderophore Production

Iron is a vital nutrient for almost all forms of life. All microorganisms known hitherto, with the exception of certain lactobacilli, essentially require iron [20]. The existence of iron in the form of Fe^{3+} at aerobic environment enables it to form insoluble hydroxides and oxyhydroxides, thus making it generally inaccessible to both plants and microorganisms [21]. Commonly, bacteria acquire iron by the secretion of low-molecular mass iron chelators referred to as siderophores which have high association constants for complexing iron. Most of the siderophores are water-soluble and can be divided into extracellular siderophores

and intracellular siderophores. In both Gram-negative and Gram-positive rhizobacteria, iron (Fe^{3+}) in Fe^{3+} -siderophore complex on bacterial membrane is reduced to Fe^{2+} which is further released into the cell from the siderophore via a gating mechanism linking the inner and outer membranes. During this reduction process, the siderophore may be destroyed/recycled [21].

Thus, siderophores act as solubilizing agents for iron from minerals or organic compounds under conditions of iron limitation [22]. Not only iron, siderophores also form stable complexes with other heavy metals that are of environmental concern, such as Al, Cd, Cu, Ga, In, Pb and Zn, as well as with radionuclides including U and Np [23]. Binding of the siderophore to a metal increases the soluble metal concentration [21]. Hence, bacterial siderophores help to alleviate the stresses imposed on plants by high soil levels of heavy metals. Plants assimilate iron from bacterial siderophores by means of different mechanisms, for instance, chelate and release of iron, the direct uptake of siderophore-Fe complexes, or by a ligand exchange reaction [24]. Numerous studies of the plant growth promotion vis-à-vis siderophore-mediated Fe-uptake as a result of siderophore producing rhizobacterial inoculations have been reported [21].

Phytohormone Production

Microbial synthesis of the phytohormone auxin (indole-3-acetic acid/indole acetic acid/IAA) has been known for a long time. Indole acetic acid (IAA) secreted by rhizobacteria interferes with the many plant developmental processes because the endogenous pool of plant IAA may be changed by acquiring IAA that has been secreted by soil bacteria [8,25]. Since, IAA acts as a reciprocal signaling molecule affecting gene expression in several microorganisms, it plays a very important role in rhizobacteria-plant interactions [25]. Moreover, down-regulation of IAA as signaling is associated with the plant defense mechanisms against a number of phytopathogenic bacteria as evidenced in enhanced susceptibility of plants to the bacterial pathogen by exogenous application of IAA or IAA produced by the pathogen [26]. IAA has been implicated in virtually every aspect of plant growth and development, as well as defense responses.

This diversity of function is reflected by the extraordinary complexity of IAA biosynthetic, transport and signaling pathways [27]. The bacterial IAA increases root surface area and length, and thereby provides the plant greater access to soil nutrients. Also, rhizobacterial IAA loosens plant cell walls and as a result facilitates an increasing amount of root exudation that provides additional nutrients to support the growth of rhizosphere bacteria [8]. Thus, rhizobacterial IAA is identified as an effector molecule in plant-microbe interactions, both in pathogenesis and phytostimulation [26]. An important molecule that alters the level of IAA synthesis is the amino acid tryptophan, identified as the main precursor for IAA and thus plays a role in modulating the level of IAA biosynthesis [18]. Strangely, tryptophan stimulates IAA production while, anthranilate, a precursor for tryptophan, reduces IAA synthesis. By this mechanism, IAA biosynthesis is fine-tuned because tryptophan inhibits anthranilate formation by a negative feedback regulation

on the anthranilate synthase, resulting in an indirect induction of IAA production [25].

However, supplementation of culture media with tryptophan increases the IAA production by most of the rhizobacteria [26]. Biosynthesis of tryptophan starts from the metabolic node chorismate in a five-step reaction encoded by the *trp* genes. The branch point compound chorismate is synthesized starting from phosphoenolpyruvate and erythrose 4-phosphate in the shikimate pathway, a common pathway for the biosynthesis of aromatic amino acids and many secondary metabolites [26].

1-Aminocyclopropane-1-Carboxylate (ACC) Deaminase

Plantgrowth promoting rhizobacteria which possess the enzyme, 1-aminocyclopropane-1-carboxylate (ACC) deaminase, facilitate plant growth and development by decreasing ethylene levels, inducing salt tolerance and reducing drought stress in plants. Currently, bacterial strains exhibiting ACC deaminase activity have been identified in a wide range of genera such as *Acinetobacter*, *Achromobacter*, *Agrobacterium*, *Alcaligenes*, *Azospirillum*, *Bacillus*, *Burkholderia*, *Enterobacter*, *Pseudomonas*, *Ralstonia*, *Serratia* and *Rhizobium* etc. Such rhizobacteria take up the ethylene precursor ACC and convert it into 2-oxobutanoate and NH_3 . Several forms of stress are relieved by ACC deaminase producers, such as effects of phytopathogenic microorganisms (viruses, bacteria, and fungi etc.), and resistance to stress from polyaromatic hydrocarbons, heavy metals, radiation, wounding, insect predation, high salt concentration, draft, extremes of temperature, high light intensity, and flooding [8]. As a result, the major noticeable effects of seed/root inoculation with ACC deaminase-producing rhizobacteria are the plant root elongation, promotion of shoot growth, and enhancement in rhizobial nodulation and N, P and K uptake as well as mycorrhizal colonization in various crops [8].

Indirect Mechanisms

The application of microorganisms to control diseases, which is a form of biological control, is an environment-friendly approach. The major indirect mechanism of plant growth promotion in rhizobacteria is through acting as biocontrol agents [8]. In general, competition for nutrients, niche exclusion, induced systemic resistance and antifungal metabolites production are the chief modes of biocontrol activity in PGPR. Many rhizobacteria have been reported to produce antifungal metabolites like, HCN, phenazines, pyrrolnitrin, 2,4-diacetylphloroglucinol, pyoluteorin, viscosinamide and tensin [14]. Interaction of some rhizobacteria with the plant roots can result in plant resistance against some pathogenic bacteria, fungi, and viruses. This phenomenon is called induced systemic resistance (ISR).

Some Fungal Biofertilizers Available on The Market

The production of commercial mycorrhizal inoculum has evolved considerably in recent years [28]. There are various types of microbial cultures and inoculants available on the market today and these have rapidly increased because of the advances in technology [29]. There are various companies worldwide marketing mycorrhiza products [30]. There are also many *Trichoderma* products as fungal

biofertilizers available in the market that are commercialized by different companies worldwide. Some of them are *Trichoderma Harzianum*, *Trichoderma hamatum* by NovaScience Co. Ltd, [30]. And also, *Penicillium bilaiae* has been formulated as a commercial product named Jumpstart[™] and was released to the market as a [31].

Success under Greenhouse and Field Condition

Trichoderma species improve mineral uptake, release minerals from soil and organic matter, enhance plant hormone production, induce systematic resistance mechanisms, and induced root systems in hydroponics [32]. For these reasons *Trichoderma* species are known as plant growth promoting fungi [33] or are increasing plant growth (biofertilization) [34]. *Trichoderma* species have therefore, successfully been used as biofungicides and biofertilizers in greenhouse and field plant production [35]. According to [36] study result; *Azospirillum* sp. a very common PGPB, have showed the same features under greenhouse and field condition.

Formulation and Inoculants Development

In inoculant formulation and development that involves an effective bacterial strain can determine the success or failure of a biological agent [37]. A microorganism which is functioning optimally under laboratory conditions might not be able to produce equivalent results under field conditions after formulation production. Once an inoculants formulation which works in situ has been developed, it must be refined to allow for the sophistication of the end-user [38]. It is imperative that the formulation remain stable during production, distribution, storage, and transportation, irrespective of whether product is new or improved. The formulation produced should also be easy to handle and apply by the end users, it should be delivered to the target site in the most appropriate manner and form, it should be able to protect the agent from various harmful environmental factors and should be able to maintain or enhance activity of the organism in the field [39]. Another important consideration is the cost-effectiveness of the formulation it should not put much pressure on the end users financially [40].

Talc Formulation

Talc or magnesium silicate ($Mg_3Si_4O_{10}(OH)_2$), occurred in the form of inert and available as raw material from soapstone industries used as a carrier for formulation development. The potential of talc to be used as a carrier was demonstrated by [41]. Rhizobacteria could survive in talc for 2 months. The Fluorescent *Pseudomonads* after storage for two months in talc mixture with 20% xanthum gum at 4°C did not decline in no. also, there are different reaserch reports that show the survival of bacterial strains in talc [42]. [43] demonstrated that application of talc-based bioformulation of *P. fluorescens* Pf1 consistently reduced the blister blight disease and increased the yield on tea plants. Further, seed treatment, soil application and seedling dip of talc-based bioformulation of Pf1 effectively reduced the sheath rot disease on rice plants under glasshouse and field conditions [44].

Press Mud Formulation

Press mud is a by-product from sugar industries that is rich in micronutrients and can minimize the utilization of synthetic fertilizers. It supplies a right type of conditions to bacteria to undertake nitrogen fixation and phosphate solubilisation. The fertilizer produced should fulfill requirements like, being free from all pathogens, harmful bacteria, weeds, easy to handle, to pack and transport. As reported by [45] the biocompost contains 25-30% organic carbon, 1.2-2.0% nitrogen, 1.5-2.0% phosphorous and 2.5-3.0% potash. This carrier increases the survival of *Azospirillum* spp. by providing suitable conditions in comparison to lignite, [46].

Vermiculite Formulation

Vermiculite is a naturally occurring layer silicate mineral [$(SiAl)Mg_3(OH)20 \cdot 10Mg0.5 \cdot nH_2O$] [47] and could also be considered as possible carriers, especially when the process of their production involves the use of specific selected strains. For example, increased amount of N and P availability in the final product can be achieved by adding N-fixing and P-solubilizing bacteria to a vermicompost [48]. Characteristics like enough space for microbial proliferation, superior aeration due to its multilamellate structure, widely availability and less cost [49], make vermiculite attractive material for the inoculant production [50].

Peat Formulations

Peat formulations have been the carriers of choice and are the most commonly used in the rhizobia inoculation industry [51]. Peat is widely available and has a long history of field trials, therefore commonly used as a carrier for PGPR, particularly for rhizobia inoculants. Peat inoculants applied to the seed as slurry is the most commonly -used method to inoculate grain legumes with rhizobia (e.g. *Bradyrhizobium* spp., *Mesorhizobium* spp., *Rhizobium* spp. etc.). Peat slurry inoculants are made using finely -milled peat that have been sterilised by gamma irradiation and these sterilised inoculants can support high concentrations of rhizobia, generally 10⁹ –10¹⁰ cells/g-1 peat at manufacture [52].

Organic Residues

since using peat as a substrate is challenged with the increase in demand and rise in cost guided to the search of an alternative substrate which possess high quality and low cost [53]. Various studies have shown that organic residues such as urban solid wastes, sewage sludge, animal manure and dung, paper waste, pruning waste, spent mushroom and even green wastes, after proper composting, can be used with very good results as container growth substrates instead of peat [53,54]. Cattle manure compost (CMC) [55], freeze-dried cells [56] and lyophilized rhizobial cells [57] can also be used as an alternatives to peat.

Lginate Formulations

Alginate is the most commonly used substance for microbial cell encapsulation. It is a natural polymeric compound made up of D -mannuronic acid and L-glucuronic acid and is available from several bacteria (*Pseudomonas* and *Azotobacter*) [58]. Alginate

beads generally have a diameter of 2-3mm, but microbeads with a size of 50 to 200 μm that can entrap up to 108 to 109 CFUg⁻¹ have also been proposed [59]. The gel-like matrix with its catalytic ability allows the cells to remain viable for longer duration. Moreover, alginate beads entrap sufficient number of bacteria [60] which shows several advantages over free cell formulations like, it protects the bacteria from biotic stresses [61] and abiotic stresses such as the inhibitory effect of toxic compounds [62], enhanced survival and improved physiological activity. This technology was primarily used to encapsulate the plant-beneficial bacteria such as *A. brasilense* and *P. fluorescens* that were used to inoculate wheat plants under field conditions [63].

Particles from Gas Saturated Solutions (PGSS)

It is used based on the application of supercritical fluid properties. It is carried out at low temperatures and uses carbon dioxide as a supercritical fluid. The final product of the process is almost spherical particles that form a free-flowing powder which can be suspended in water. The possibilities of the PGSS process have already successfully been demonstrated for several solids and liquids [64].

Bacterial Biofilms as A Possible Carrier

Two types of biofilms are employed in that case: biofilms growing onto inert supports (charcoal, resin, concrete, clay brick, sand particles) in which biofilms grow all around the particles, and the size of the biofilm particles grows with time usually to several mm in diameter and biofilms that are formed as a result of aggregate formation also called granular biofilm which may take from several weeks to several months [65]. Wheat seedlings inoculated with biofilm-producing bacteria exhibited an increased yield in moderate saline soils [66].

Bionanotechnology

Applications which employ nanoparticles made of inorganic or organic materials could also provide new avenues for the development of carrier-based microbial inocula [67]. The use of nanoformulations may enhance the stability of biofertilizers and biostimulators with respect to desiccation, heat and UV inactivation.

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