**Bioinoculants: An Ecofriendly Approach towards Artificial Fertilizers in Sustainable Agriculture"**

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Abstract:

Bioinoculants, also known as microbial biostimulants, are gaining widespread attention in modern agriculture due to their potential to enhance crop productivity and improve soil health while reducing the reliance on synthetic chemicals. The different types of bioinoculants, includes nitrogen-fixing bacteria, phosphate-solubilizing microorganisms, plant growth-promoting rhizobacteria (PGPR), mycorrhizal fungi, and other beneficial microbes. The role of bioinoculants in fostering plant-microbe interactions, particularly within the rhizosphere, shows how these interactions enhance nutrient uptake, promote root system development, and contribute to the overall health and resilience of crops. Moreover, the potential synergistic effects of combining multiple bioinoculant strains to optimize their benefits are explored.

Keywords: bioinoculant, plant growth promoting bacteria, rhizobium

**Introduction:**

Bacteria that colonize the rhizosphere and plant roots, and enhance plant growth by any mechanism are referred to as plant growth-promoting rhizobacteria (PGPR). Another major benefit of PGPR is to produce antibacterial compounds that are effective against certain plant pathogens and pests (Dey et al., 2004; Herman et al., 2008; Minorsky, 2008). Bioinoculants are eco-friendly microorganisms having a variety of products commonly utilized for improving the potential of soil and providing the nutrient requirements to the host plant. The usage of chemical fertilizers is not beneficial because it affects the soil microbial communities on large scale. The utilization of PGPR in the bioinoculant form imparts successfully sustain agricultural yield production and such formulated products contain living microbial cells of bioinoculants that also helps in seed treatment and enhances the mobilization process of nutrients by the low-cost process(chaudhary et al., 2020).

The uncontrolled use of chemical fertilizers and pesticides to increase crop yields is of concern in terms of environmental deterioration, wildlife conservation, and human health. Simultaneously, due to inadequate land management and different environmental factors, soil degradation has intensified through drought, flooding, high temperatures, and soil salinity. An environmentally friendly alternative that can address these issues is the use of biofertilizers as plant growth–promoting rhizobacteria (PGPR) (Basu et al. 2021). PGPRs are free-living bacteria that can enhance plant growth and/or provide protection against biotic or abiotic stresses by colonizing roots (Kloepper and Schroth 1978). These microorganisms have long been considered a promising tool, but their mechanisms of action and performance under real field conditions are still a matter of research. The application of plant growth promoting microorganisms has proven to be a greener approach suitable to improve cultivation of several plant species even under stressful environmental conditions such as drought, salinity, and high temperatures.

**Types of bioinuculent**

Bio inoculent are formulations of beneficial microorganisms that are applied to seeds, seedlings, or soil to enhance plant growth and productivity. Rhizobium bioinoculants are used to improve nitrogen availability in the soil for leguminous crops, thereby reducing or eliminating the need for synthetic nitrogen fertilizers. Turner, 2013; Lebeis, 2014; Smith, 2015).

Microbes possess the ability to assimilate and acquire vital nutrients from the soil, facilitating their availability to plants. They contribute to the improvement of soil physicochemical properties and have the capacity to modulate various aspects of plant biology, including the production of secondary metabolites, antibiotics, plant hormones, and different signal compounds. Additionally, microbes secrete a range of biostimulants that play significant roles in influencing physiological and metabolic activities in plants. (Nelson,2015 Leach,2017,  Massalha,2017)

**Rhizobium:**

Rhizobium is a genus of bacteria that forms a symbiotic relationship with leguminous plants, such as peas, beans, and clover. These bacteria infect the roots of these plants and form specialized structures called nodules, where they convert atmospheric nitrogen into a form that the plant can utilize. This process is known as biological nitrogen fixation. Performance of microbial inoculants as Rhizobium inoculants under field conditions is the principal criterion of selection as a commercial biofertilizer.

According to Lucy *et al.* (2004), soil is a highly heterogeneous and unpredictable environment and anticipated results are often difficult to achieve. For this reason, continuous use of biofertilizers enables the microbial population to remain and build up in the soil and helps in maintaining soil fertility contributing to sustainable agriculture (Choudhury and Kennedy 2004). In most cases, application of Rhizobium in pot trials and field experiments showed a statistically significant increase in several crop production parameters such as grain production, root length, leaf length, or plant weight (Naher et al. 2009; Mehboob et al. 2011; Yanni and Dazzo 2010). The colonization of Mayang Segumpal rice by Rhizobium sp. (SB16) increased plant biomass by 36% over the non-inoculated control and the higher tissue nitrogen content by 4.47% (Naher et al. 2009). Abera et al. (2016) reported in Toke Kutaye (western Ethiopia) significantly higher mean grain yield of maize after the application of half recommended nitrogen fertilizer following faba bean precursor crop with rhizobium inoculation. Similarly, Saini et al. (2004) suggested that for maximum crop yield of sorghum (Sorghum bicolor L.) and chickpea (Cicer arietinum L.), only 50% of the required fertilizer might be supplied along with bioinoculants (Rhizobium, or A. brasilense, Bacillus megaterium, and Glomus fasciculatum)

Recently Canto *et al*. (2023) studied indigenous drought tolerant rhizobium strains as promising biostimulants for common bean in Northern Spain in which the nine bacteria strains evaluated, three were found to be highly efficient under drought (namely 353, A12 and A13). These strains sustained high infectiveness (nodulation capacity) and effectiveness (shoot biomass production) under drought, even surpassing the plants inoculated with the CIAT899 reference strain, as well as the chemically N-fertilized plants. Inoculation of pulses with biostimulants such as rhizobium strains with high nitrogen fixation efficiency and drought-tolerance, has emerged as a promising and sustainable production strategy.

**Azospirillum**

Azospirillum is a genus of bacteria that is commonly used as a bioinoculant in agriculture. These bacteria are beneficial plant growth-promoting rhizobacteria (PGPR) that can establish a symbiotic relationship with a wide range of plants, including cereals, vegetables, and grasses. While Azospirillum bacteria are not capable of fixing atmospheric nitrogen like Rhizobium, they can promote nitrogen availability in the soil through other means. Azospirillum can solubilize and mineralize organic nitrogen in the soil, making it more accessible to plants. Additionally, they can enhance the uptake of nitrogen from synthetic fertilizers or organic sources.

The inoculation of plants with Azospirillum result in a significant change in various growth parameters in different cereals such as an increase in plant biomass, nutrient uptake, tissue N-content, plant height, leaf size, tiller numbers, root length, and volume (Salantur et al., 2006) Increase in yield accompanied by increased nitrogen concentration due to Azospirillum inoculation was attributed to enhanced nitrogen fixation or enhanced assimilation by plants (Wani and Lee, 1991). Increase in N content, dry weight of shoot, roots, and plant height of Setaria italic was documented (Rafi and Charyulu, 2016). Inoculationof Z. mays and Sorghum bicolor with A. brasilense strain Cd or Sp-7 significantly enhanced (30%–50% over controls) the uptake of mineral ions and also 20%–30% increase in shoot dry weight was observed (Lin et al., 1983).

**Mycorhizae:**

Mycorrhizae, specifically arbuscular mycorrhizal fungi (AMF), are often used as bioinoculants in agriculture and horticulture. Mycorrhizal fungi have a remarkable ability to extend their fine hyphae into the soil, greatly increasing the effective root surface area for nutrient absorption. They can efficiently extract nutrients, such as phosphorus, nitrogen, potassium, and micronutrients, from the soil and deliver them to the host plant. This improves nutrient uptake, especially in soils with low nutrient availability. Mycorrhizal fungi form a symbiotic relationship with the roots of most plant species, including trees, shrubs, and agricultural crops. This symbiosis benefits both the fungi and the plants involved.

Baisru *et al* (2021) examined 68 mycorrhizal products from 28 manufacturers across Europe, America, and Asia were on varying properties such as physical forms, arbuscular mycorrhizal fungal composition, number of active ingredients, claims of purpose served, mode of application, and recommendation. In whuch they that 100% of the products are based on the Glomeraceae of which three species dominate among all the products in the order of Rhizophagus irregularis (39%), Funneliformis mosseae (21%), Claroideoglomus etunicatum (16%). Rhizophagus clarus is the least common among all the benchmark products. One third of the products is single species AMF and only 19% include other beneficial microbes. Of the sampled products, 44% contain AMF only while the rest are combined with varying active ingredients. Most of the products (84%) claimed to provide plant nutrient benefits.

Faye et al (2013) 12 arbuscular mycorrhizal fungi (AMF) inoculants were evaluated in a two-step experiment under greenhouse conditions using maize. Six weeks after planting, seven inoculants increased root colonization levels compared with control soil, while only three inoculants increased slightly the shoot biomass of maize plants.

**Plant growth promoting bacteria:**

**Phosphate-solubilizing bacteria (PSB):**

These bacteria convert insoluble forms of phosphorus in the soil into soluble forms that plants can absorb. PSB produce organic acids, such as citric acid and gluconic acid, which can chelate phosphorus, releasing it from minerals like rock phosphate. Inoculation of seed with P-solubilizing microorganisms is a promising technique which may alleviate the deficiency of phosphorus. Examples of PSB include species of Bacillus, Pseudomonas, and Enterobacter.

Phosphate solubilizing Bacillus sp significantly enhanced the seed cotton yield and plant height (Table 2). The highest seed cotton yield was produced with Bacillus inoculation (1733.3 kg ha-1) at 90 kg P ha-1. Bacterial inoculation produced higher seed cotton yield at all P levels compared to their respective control. Percent increase in seed cotton yield with inoculation was 8.08, 7.93 and 7.57% at 30, 60 and 90 kg P ha-1, respectively. PGPR having the potential of phosphate solubilization enhanced the growth hormone production, availability of phosphorus and rate of nitrogen fixation (Ponmurugan and Gopi, 2006)

Panhwar et al (2014) found that PSB populations were higher in rhizosphere than non-rhizospheric soil and the highest population was found in PS and Pikovskaya, while the lowest was found in PA media plates. The highest P solubilizing activity (69.58%) was found in PSB9 strain grown in NBRIP plate. Isolated PSB were able to produce different organic acids and growth hormone such as IAA. A number of PSB isolates belong to the Bacillus sp. and proved for the antagonistic effect against R. solani (sheath blight) even though most of the isolated strains can grow in nitrogen, free semi-solid medium and able to produce siderophore.

Liu et al (2015) The NBRIP liquid medium culture showed four PSB strains lowered medium pH (<4.3) and released WS-P up to 523.69 mg/l with three days incubation and Krome3 strain dissolved 95.3% tricalcium phosphate added after 35 days incubation. Incubation of PSB in a sandy soil showed that PSB increased WS-P, but not Mehlich-3 P.

**Silicate-solubilizing bacteria:**

Silicate-solubilizing bacteria solubilize insoluble forms of silica in the soil, releasing plant-available silicon. Silicon plays a role in plant defense mechanisms, enhancing disease resistance and stress tolerance. A group of bacteria called silicate-solubilizing bacteria (SSB) is involved in the conversion of silicates into soluble silica. These groups of bacteria produce silicase, an enzyme responsible for the conversion of silicates into soluble silica, making Si available for plant uptake.

Sulizah et al (2018) isolated five silicate solubilizing bacteria OS4, OS5, OS7, OS12 and OS13. The highest Solubilizing Index was gained by OS7 on 1,10, while the highest silicate concentration was solubilized by OS12 on 1,053 ppm in Bunt and Rovira broth. Bacteria such as Bacillus, Pseudomonas, and Paenibacillus have been found to solubilize silicate minerals.

**Sulfur-solubilizing bacteria (SSB):**

Sulfur is an essential nutrient for plant growth, and SSB help in solubilizing insoluble sulfur compounds in the soil, making it available for plants. These bacteria produce enzymes that convert insoluble sulfur into soluble forms. Examples of SSB include Thiobacillus and Rhodococcus species.

Malviya et al (2022) isolated Sulfur-oxidizing bacteria (SOB) from coal mines out of thirteen two isolates outperformed as a microbial inoculants viz., *S. maltophilia* DRC-18-7A closely followed by S. pavanii DRC-18-7B plants treated with microbial inoculants induce an early formation of secondary and tertiary roots in the pigeonpea compared to the untreated control.

**Potassium-solubilizing bacteria (KSB):**

KSB are capable of solubilizing insoluble potassium minerals in the soil, releasing potassium ions that can be taken up by plants. These bacteria produce organic acids that aid in the solubilization process. Bacillus, Pseudomonas, and Azotobacter are examples of KSB.

Supanjani *et al.* (2006) reported that inoculation of phosphorus- and potassium-solubilizing bacteria on Capsicum annuum along with addition of P- and K-containing rock materials increased P availability from 12 % to 21 % and K availability from 13 % to 15 % in the soil as compared with control and subsequently improved N, P, and K uptake in the crop

Inoculation of seed or seedling with microphos biofertilizers can provide 30 kg P2O5/ha (Ghumare *et al.* 2014). Kumar and Kumawat (2014) reported that summer mung bean cv. T-1 sown with combined use of chemical fertilizer (10 kg N and 20 kg P2O5/ha), 50% N as vermicompost (10 kg/ha), and biofertilizers (20  g PSB/kg seed) enhances the crop prouction, economics, and soil fertility.

**Zinc-solubilizing bacteria (ZSB) :**

ZSB are a group of beneficial microorganisms that have the ability to solubilize insoluble forms of zinc in the soil, making it available for plant uptake. These bacteria play an important role in improving zinc availability, which is essential for plant growth and development.

Ramarethinam and Chandra (2005) recorded significantly increased egg plant yield, plant height and K uptake compared to control in a field experiment due to inoculation of potashsolubilizing bacteria Frateuria aurantia. Mikhailouskaya and Tchernysh (2005) reported the effect of inoculation of K-mobilizing bacteria on severally eroded soils which were comparable with yields on moderately eroded soil without bacterial inoculation that resulted in increased wheat yield upto 1.04 t/ha

The important crops such as maize (,Goteti et al 2013, Hussain et al., 2015, Biari et al 2008, Omari et al 2016), rice (Vaid et al., 2014, Tariq et al., 2007, Zeb et al., 2018, Gontia-Mishra et al., 2017, . Idayu et al., 2017) and wheat (Ramesh et al., 2014, Kumar et al., 2017 Singh et al., 2017, Rana et al., 2012, Kalinowski et al., 2000, Khande et al., 2017) have been studied extensively for Zn biofortification in response to ZSB inoculants as the grain parts from these crops offer the most important staple foods on a broad scale worldwide. A potential ZSB microbial strain, namely Bacillus sp. enhanced the Zn translocation (%) in two different Basmati rice varieties, i.e., 22–49% (for Basmati-385) and 18–47% (for Super-Basmati Rice) (Shakeel et al. 2015). The study by Wang *et al*. (2014) illustrated the role of “*Enterobacter* sp. SaCS20” and “Sphingomonas sp. SaMR12” in improving the Zn content in polished rice by 11.2% and 13.7%. Bacterium “Rahnella sp. JN6” improved the plant growth and increased Zn accumulation in Brassica napus (oilseed rape) in pot experiments (2013).

There are many zinc-solubilizing microbes, such as *B. subtilis, Thiobacillus thioxidans and Saccharomyces sp*., which are capable of solubilizing soil zinc. Such microbial inoculants may be used as biofertilizers for solubilizing soil zinc (Raj, 2007). Such Zn-solubilizing bacteria (Bacillus sp.) may be used as biofertilizers where soil zinc is found in higher concentration in various insoluble forms such as zinc oxide (ZnO), zinc carbonate (ZnCO3) and zinc sulphide (ZnS) in place of costlier zinc sulphate.

**Conclusion:**

Mineral-solubilizing bacteria come from a variety of phyla, including Ascomycota, Actinobacteria, Basidiomycota, Bacteroidetes, Chlorobi, Cyanobacteria, Chlorophyta, Euryarchaeota, Firmicutes, Gemmatimonadetes, Mucoromycota, Proteobacteria, and Tenericutes, between others. The release of plant growth regulators, the dissolving of phosphorus, potassium, zinc, selenium, and silicon, as well as the biological nitrogen fixation and production of siderophores, ammonia, hydrogen cyanide, hydrolytic enzymes, and bioactive compounds/secondary metabolites constitute the various ways that mineral solubilizing microbes (MSMs) indirectly or directly stimulate plant growth and development.

The challenge of feeding the expanding global population can be handled with the aid of biofertilizers at a moment in which agriculture has to cope with a number of environmental challenges. Understanding the beneficial effects of biofertilizers and adopting them into conventional farming practises are crucial.

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Type of bioinoculant

Sulphur solubilizing bacteria

Zinc solubilising bacteria

Potassium solubilising bacteria

Silica solubilising bacteria

Phosphate solubilising bacteria