**REVIEW ARTICLE** 



# Microbial Consortia: Promising Tool as Plant Bioinoculants for Agricultural Sustainability

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### Abstract

In the present scenario, growing population demands more food, resulting in the need for sustainable agriculture. Numerous approaches are explored in response to dangers and obstacles to sustainable agriculture. A viable approach is to be exploiting microbial consortium, which generate diverse biostimulants with growth-promoting characteristics for plants. These bioinoculants play an indispensable role in optimizing nutrient uptake efficiency mitigating environmental stress. Plant productivity is mostly determined by the microbial associations that exist at the rhizospheric region of plants. The engineered consortium with multifunctional attributes can be effectively employed to improve crop growth efficacy. A number of approaches have been employed to identify the efficient consortia for plant growth and enhanced crop productivity. Various plant growth-promoting (PGP) microbes with host growth-supporting characteristics were investigated to see if they might work cohesively and provide a cumulative effect for improved growth and crop yield. The effective microbial consortia should be assessed using compatibility tests, pot experimentation techniques, generation time, a novel and quick plant bioassay, and sensitivity to external stimuli (temperature, pH). The mixture of two or more microbial strains found in the root microbiome stimulates plant growth and development. The present review deals with mechanism, formulation, inoculation process, commercialization, and applications of microbial consortia as plant bioinoculants for agricultural sustainability.

### Abbreviations

ACC	1-aminocyclopropane-1-carboxylate
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- K Potassium
- N Nitrogen
- P Phosphorus

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- PGPPlant growth promotionPGPRPlant growth-promoting rhizobacteriaPSMsPhosphorus-solubilizing microbesZnZinc
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### Introduction

Addressing global food insecurity is a persistent challenge that is expected to exacerbate due to climate change, rapid population expansion, and a shortage of arable land. The significant and anticipated growth in global population will place substantial pressure on food security. In an attempt to meet the rising demand for food, there has been an unbalanced utilization of agrochemicals, including fertilizers and pesticides for crop production. In light of the growing population and dwindling natural resources, there is a pressing requirement to enhance agricultural productivity in a sustainable and environmental friendly manner. Traditional agricultural methods heavily rely on chemical fertilizers to boost productivity, but this practice poses risks to agro-ecosystems, including contamination of the food chain, deterioration of soil quality, and water pollution [1]. Plant growth frequently takes place in various challenging environments, including soils with varying degrees of acidity and alkalinity. The pH of the soil, controlled by hydrogen ion concentration, plays a crucial role in regulating the chemical properties of nutrient colloidal solutions for plants. When soil pH surpasses certain thresholds, it can lead to various stresses in plants, including hydrogen ion toxicity and imbalances in nutrients, resulting in toxicities or deficiencies. In agriculture, one approach to address these issues has been to breed plants for stress tolerance and combine these efforts with appropriate agronomic practices to manage such challenges [2]. It is crucial to make transition toward sustainable agricultural practices, such as the utilization of plant growth promotion (PGP) microbes as an alternative to traditional chemical fertilizers to address these challenges. This shift can contribute to the restoration of agro-ecosystems.

All plants require a variety of minerals from their surroundings in order for their vegetative and reproductive tissues to grow and develop appropriately. These minerals have a variety of uses, such as being ionized species that balance charges in cellular compartments, cofactors in enzyme activities, osmotic solutes required to maintain the right water potential, and structural elements of macromolecules [3]. Based on the proportional amounts required for plant growth, minerals can be classified into two types. The macronutrients are sulfur (S), calcium (Ca), magnesium (Mg), phosphorous (P), nitrogen (N), and potassium (K). In plants, these elements are often present in amounts higher than 0.1% of dry tissue weight. Iron (Fe), zinc (Zn), manganese (Mn), copper (Cu), boron (B), chlorine (Cl), molybdenum (Mo), and nickel (Ni) are among the currently recognized micronutrients; these are often found at concentrations less than 0.01% of dry tissue weight.

The PGP microorganisms refers to any type of microbe, including bacteria, fungi, algae, and actinomycetes, that

promotes plant development through direct or indirect methods [4]. They play a vital role for crop production and protection for sustainable agriculture. They boost diversity and interaction with other beneficial microbes, enhance soil fertility, boost crop productivity, prevent the growth and infectious activity of possible diseases, and overall preserve the sustainability of the systems. The real aspect of these interactions in the environment, where a variety of microbial species can occur, is overlooked despite the fact that this has improved our understanding of plant–microbe interactions [5].

The term microbial consortium is a group of two or more beneficial microorganism that work together and contain diverse array of microbes that exhibited PGP through a variety of mechanisms and enhance activity against other pathogens [6]. Various studies have reported that inoculation of microbial consortium performs better task than individual microbial strain [7]. Numerous studies have shown that the effect of biofertilizer containing two or more microbial strains also referred to as co-inoculation or consortium is more advantageous than the application of a single microbial strain. The PGP microbial formulation used as biofertilizer generally uses a single microbial strain. However, inconsistent outcomes were observed when this biofertilizer was applied to the soil, because it is difficult for alone strain to break down complex substances like cellulose, execute two-step or multi-step reactions, and maintain stability in an unstable environment. Microbial consortia are a great alternative to improve plant growth, increase crop productivity, soil properties and biodiversity, and reduce the chemical inputs [8]. The mixture of two or more microbial strains found in the root microbiome stimulates plant growth and development. The present review deals with mechanism, formulation, inoculation process, commercialization, and applications of microbial consortia as plant bioinoculants for agricultural sustainability.

# Mechanism of Plant Growth Promotion by Microbial Consortia

Plant growth-stimulating microbes facilitate the plant growth by two types of mechanisms either direct and or indirect mechanisms. Direct mechanism of microbes stimulates the plant growth directly through production of various plant growth stimulators, such as auxin, cytokinin, and gibberellin; iron sequestration through the production of siderophores; and availing the macro- and micronutrients through fixation, chelation, and solubilization. The indirect mechanism facilitates the plant growth by protecting the plants from biotic stress exerted by pathogens (bacteria, fungi, virus, and oomycetes) and pest (insects) [9]. Production of cell wall-degrading enzymes (chitinase, amylase, protease, xylanase, and pectinase), antibiotics hydrogen cyanide, siderophore, and 1-aminocyclopropane-1-carboxylate (ACC) deaminase activity [10, 11] (Fig. 1).

### **Absorption of Nutrients from Soil**

The appropriate growth of plants requires different types of macro- (nitrogen, potassium, and phosphorus) and micronutrients (iron and zinc). Plants assimilate these nutrients from soil and in soil various forms of nutrients, i.e., soluble, and insoluble forms. In between both the forms, plants could

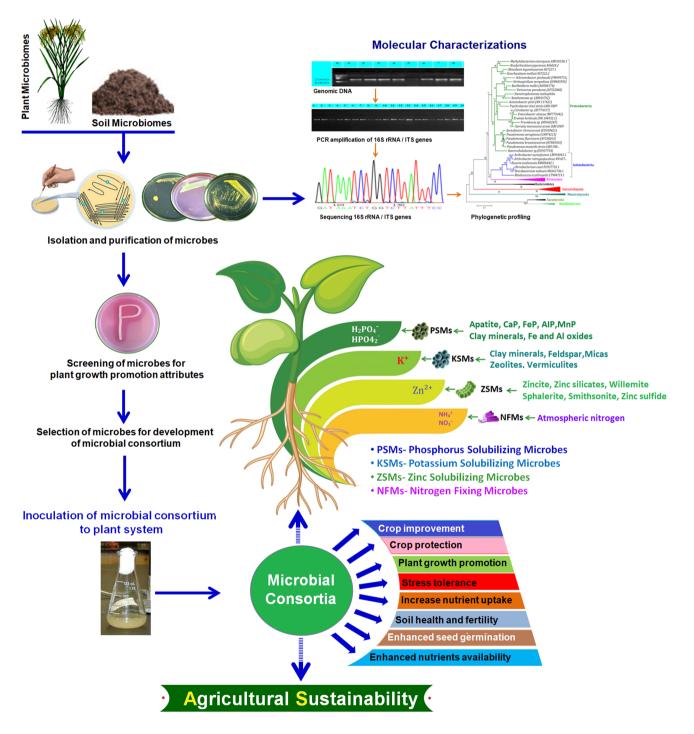


Fig. 1 Mechanism of microbial consortia for enhancement of crop productivity for agricultural sustainability

absorb only soluble form of nutrients. Most of the soil in the world lacks the soluble form of nutrients which is one of the main reasons for the plant growth depletion and low productivity. Microbes from soil and plant niches could convert the insoluble form of nutrients to soluble form via fixation and solubilization mechanism. Fixation process avails the nitrogen nutrients, whereas solubilization process coverts insoluble form to soluble form of phosphorus, potassium, and zinc [12–14].

#### **Nitrogen Fixation**

Nitrogen is essential macronutrients as it is the most important structural nutrient of the cell components (DNA, protein, and RNA) of plant [15]. Plant absorbs nitrogen from soil in the reduced form, i.e., ammonia, and this form of nutrient is in low concentration. Although, nitrogen is the most abundant nutrient in the atmosphere in the form of dinitrogen gas which is unavailable for plant use. To fulfill the plant requirement of nitrogen, urea was being utilized by the farmers which was prepared synthetically through Haber–Bosch process. The continue practice of urea in the fields has resulted in the serious damages to the environment and the humans [16].

As a substitute, microbes with nitrogen fixation capability could be used for reducing the dinitrogen into ammonia through the process of biological nitrogen fixation. Microbes with the attribute of nitrogen fixation have been reported for having specialized gene named *nif* which codes for a complex system of enzyme nitrogenase [17]. The complex system of enzyme nitrogenase was elucidated into two different metalloenzyme components consisting of iron protein dinitrogenase reductase (provides electron with high reducing power) and a metal cofactor dinitrogenase (uses electrons to reduce the dinitrogen into ammonia). On the basis of metal cofactor, three types of nitrogen-fixing systems were identified, namely Mo-nitrogenase (most abundantly found), V-nitrogenase, and Fe-nitrogenase, which are present in among various genera of bacteria [18].

In a report, nitrogen-fixing bacterial strain EU-A3SNfb *Rahnella* sp. was combine with P and K-solubilizer, namely *Bacillus tropicus* EU-ARP-44 and *B. megaterium* EU-ARK-23, respectively. The combined effect of these strains significantly exhibited growth and physiological parameter of *Aegilops kotschyi* and wheat crop under greenhouse and field conditions [19]. In an another report, a microbial mixture of *Erwinia* sp. EU-B2SNL1 (N-fixer), *Chryseobacterium arthrosphaerae* EU-LWNA-37 (P-solubilizer), and *Pseudomonas gessardii* EU-MRK-19 (K-solubilizer) were inoculated on barley. The result showed that the combined effect of these strains increased physiological parameters and overall growth and was found to be more efficient as compared to a single inoculum [20]. A study by Kour et al.

[21] reported N-fixing and K-solubilizing bacterial strains, namely *Acinetobacter guillouiae* (EU-B2RT.R1) and *Acinetobacter calcoaceticus* (EU- LRNA-72) inoculated in onion. The result revealed that co-inoculation of these strains positively impacted shoot length, root length, biomass, phenolic, flavanoids, total soluble sugars, and chlorophyll content.

#### **Phosphorus Solubilization**

Phosphorus (P) is one of the indispensable macronutrients required for the various key metabolic processes in plants life. Energy generation, macromolecules biosynthesis, cell division, photosynthesis, signal transduction, member integrity respiration, and fixation of nitrogen are the various significant processes in which phosphorus nutrient plays an important role. Plants acquire P from the soil present in the top layer but the amount for plant uptake is only 0.1% of the total available amount (50 to 3000 mg  $kg^{-1}$ ), due to cations precipitation, absorption, immobilization, and organic form interconversion [22]. Being an essential nutrient farmer has totally relied on phosphatic fertilizers and its excessive utilization had precipitated the soil and accumulated the heavy metals. The precipitation and heavy metal accumulation caused various determinantal effects on fertility soil, animal, and consumers health [23]. Looking at the health hazards of using chemically processed fertilizers, an eco-friendly approach exigency is very important. Phosphorus-solubilizing microbes (PSMs) have been known as an appropriate approach without any cons [24].

Phosphorus-solubilizing microbes solubilize the P with the help of solubilization mechanisms and they are converted in to soluble form with the help of several processes, namely, production of protons, organic acids, inorganic acid, siderophores, hydrogen sulfide (H<sub>2</sub>S), and extracellular enzymes [12]. Organic acid production releases the soluble form of P by lowering the pH and complexation the metal ion. Microbes produces various organic acids like malic acid, lactic acid, lactic acid, gluconic acid, oxalic acid, tartaric acid, and 2-ketogluconic acid which helps in releasing the P [25]. The production of inorganic acids (hydrochloric acid, carbonic acid, nitric acid, sulfuric acid, and H<sub>2</sub>S) solubilizes P with low efficiency as compared to organic acid. Protons production also lower the pH of the soil aids the dissolution of phosphorus. The production of exopolysaccharides releases the soluble form by forming the complex with metal ions present in soil and this extrapolated as a mean solubilization. Microbes could also solubilize the P through enzymatic actions of phytases and phosphonatases and C–P lyases [14].

Numerous microorganisms from diverse habitats have been reported for solubilizing the P nutrient and found to enhance the growth of the host plants upon inoculation [26]. In a study Devi et al. [27] reported that phosphorus solubilizer and nitrogen-fixing bacterial strain identified as *Pseudomonas gessardii* and *Erwinia rhapontici* were evaluated on *Amaranthus* crops. The study showed that co-inoculation of both of strain significantly enhances plant growth and physiological parameter such as content of chlorophyll, carotenoids, sugar, phenolics, and flavonoids. In a different report, three efficient bacterial strains identified as *Bacillus subtilis* (P-solubilizer), *Bacillus amyloliquefaciens* (K-solubilizer), and *Pseudomonas extremorientalis* (N-fixer) were evaluated on pearl millet as single inoculum and as bacterial consortia promote physiological parameter including content of sugar, phenolics, flavonoids, chlorophyll, carotenoids, and overall growth as compared to single inoculum [28]

### **Potassium Solubilization**

Potassium is the third essential macronutrient for plants which plays role in physiological and metabolic processes, such as growth, photosynthesis, accumulation of sugar, and regulates the rate of photosynthetic carbon assimilation [29]. Additionally, K plays roles like root growth, high yield seed development, metabolism of organic acids, fats, carbohydrates, nitrogenase compounds, biosynthesis of proteins, stomata regulation, water uptake and could mediate resistance against biotic stresses caused by various pathogens [30]. Plant assimilates the K from soil in the soluble form, i.e., K<sup>+</sup> ion which is present in limited amount and about 80-90% of K is exist insoluble form including biotite, illite, orthoclase, muscovite, zeolite, glauconite, chlorite, and vermiculite [31]. To fulfill the limitation of K, potash fertilizer is widely used which have proven to be hazardous to consumers and environment. To overcome this constraint, potassium-solubilizing microbes are one of the eco-friendly approaches which could solubilize the insoluble form and release the K<sup>+</sup> ion. Likewise, solubilization P, insoluble form of K is dissolute by the production of organic acids, extracellular enzymes, and exopolysaccharides [32].

In a report, three bacterial strains namely *Halomonas* aquamarina (K-solubilizer), Erwinia persicina (P-solubilizer), and Pseudomonas extremorientalis (N-fixer) were evaluated on chilli. The study showed the combined effect of these strains significantly improved plant growth parameters such as root length, shoot length, fresh weight, dry weight, and physiological parameters, including content of phenolics, flavonoids, sugar, chlorophyll, and carotenoids as compared to single inoculum [33]. In a different report, bacterial strains identified as Bacillus sp. (K-solubilizer), Pseudomonas marginalis (P-solubilizer), and Stenotrophomonas rhizophila (N-fixer) were inoculated on foxtail crop. The study revealed that co-mixture of these strains exhibited growth of plant as compared to single inoculums, chemical fertilizer, and untreated control [34].

### **Zinc Solubilization**

Zinc is the most significant micronutrient among all other which plays crucial role in regulation of cofactors that activates wide range of enzymes. Additionally, this nutrient also helps in the maintaining the cellular membrane integrity, synthesis of proteins, regulating of auxin biosynthesis, and pollen production. Plants uptake zinc from the soil in form of zinc ions and the concentration of Zn ion is very less. Zinc sulfate, zinc oxide and zinc carbonate are in very high concentration which cannot be assimilated by the plants, due to which plants have zinc deficiency which cause problem, such as yellowing of the young plants, necrosis, shortened internode, sterility of pollens, and spikelet [35]. Zinc solubilization mechanism exhibited by zinc-solubilizing microbes could alleviate the deficiency of the nutrient by converting the insoluble form to soluble form. Microbes could solubilize the insoluble form of zinc either producing various organic acids such as 2-ketogluconic acid and 5-ketogluconic acid which lowers of the pH or by metabolites that form a complex with zinc ion. In literature, various zinc-solubilizing microbes from diverse niches have been reported which also helps in the plant growth promotion [36].

In another investigation, zinc-solubilizing bacteria *Streptomyces venezuelae* and *Klebsiella aerogenes* were found to enhance Zn biofortification in the wheat upon inoculation with ZnO nanoparticles and improving its growth [37]. In a finding, potential zinc-solubilizing bacterial strain identified as *Bacillus* sp. SH-10 and *Bacillus cereus* SH-17 were evaluated on rice as individually or as combine mixture. The mixture of bacterial strain improve yield and Zn content as compare to chemical fertilizer [38].

# Formulation and Inoculation Process of Microbial Consortia

The primary objective when contemplating plant inoculation with PGP microbes is to identify the most efficient bacterial strain or microbial consortia that exhibits multifarious plant growth-promoting attributes and can achieve the desired outcome for the specific target crop. Subsequently, the next step involves creating a tailored inoculant formulation for the target crop, devising a practical application method, and considering the limitations of the growers. The main practical features of inoculants expected by the grower are that the inoculant has to be compatible with routine field practices. Secondarily, important features of microbial consortia are compatibility with the seeding equipment at the time of seeding, ability to work under different field conditions, types of soil, tolerance of abuse during storage, ability to help prolong survival of the inoculated microbe for the time needed by the plant, shelf life that lasts more than one season, reproducible results in the field, and human, animal, and plant safety by eliminating the use of hazardous materials [39].

The formulation of a microbial consortium involves the following steps: preparing the inoculums; adding additives; choosing a carrier; sterilizing the carrier material; upscaling; performing quality control processes; and packaging the product appropriately with the best distribution channels. Understanding the complexities of microbial interactions in their natural environments is the biggest challenge in designing the consortium. As a result, it will become easier to design consortia when you have a solid understanding of metabolic pathways, compatibility of microbes and their limitations [40]. The process of discovering and characterizing consortia involves a bottom-up selection approach. This approach encompasses the collection of microbial cultures and the analysis of their properties through culture-dependent screening techniques [41]. Screening tests primarily depend on specific microbial functions, such as nitrogen fixation, antibiotic production, siderophore production, phosphate solubilization, plant hormone production, and ACC deaminase activity.

The most promising microbial consortium is then assessed in greenhouse conditions, and further testing is carried out in the field following a bottom-up approach. It is worth noting that while many microbial strains may demonstrate success in laboratory and greenhouse settings, they may encounter challenges when it comes to enhancing the suboptimal plant microbiome in the field. Laboratory testing may offer limited insights. For instance, a Pseudomonas strain that exhibited antagonistic activity against Phytophthora infestans when developed in co-culture with another Pseudomonas strain, lost its biocontrol activity under field conditions [42]. Top-down strategies enable the study of microbiome properties at a molecular level, allowing for the selection of PGP-consortium candidates based on this molecular information. This has become possible through the direct identification of core and satellite microbiota in environmental samples, relying on single amplicon variants obtained through high-throughput sequencing of nucleic acids [43]. Formulations are essential to maintain the longterm viability of microbial cells during storage and to provide an adequate number of viable cells for field-grown plants. Unfortunately, there is a shortage of formulations available for many microbes, particularly Gram-negative bacteria [44].

There are only few different ways to distribute PGP microbes as microbial consortium in the field. Farmers are not keen on purchasing specialized machinery to be used for microbial-based products. Therefore, prepared microbial inoculation should be easily applied using common farming equipment and simple procedures [45]. Formulated microbial consortia as biofertilizers can be used for a variety of

applications, including seed inoculation with powder formulations, mixing water and peat powder, dry fertilizers mixed with the seeds, soil application and seedling root dip, and suspending the biofertilizer in water along with seeds [46].

### Soil Inoculation

When microorganisms are put directly into the soil, a process known as soil inoculation, they face competition from native microbes that have already adapted to the local environment and outnumber the microbial inoculums [8]. It is possible to significantly influence the soil's microbiological balance and improve the environment for plant growth and protection using inoculants of mixed cultures of beneficial microorganisms [47]. Granules or liquid inoculants can be added to the seedbed to inoculate the soil. The possibility that part of the inoculants will be lost during seeding machinery and seeding is decreased when the soil is inoculated. Small seeds are more benefited by soil inoculation than by seed-coat inoculation because they can be exposed to higher quantities of inoculants [39]. A microbial consortium solution is introduced to the soil during soil soaking as closely as feasible to the host root. This is essential because it is in the rhizosphere that the PGP microbes will be able to carry out various essential tasks for supporting plant growth promotion, such as solubilization of phosphate, potassium, and zinc, and synthesis of siderophores and phytohormones [48]. In an investigation, [49] reported that by introducing inoculums through soil, Bacillus improves plant growth. The plant growth-promoting (PGP) microbes inoculated through soil improved the growth of Ranunculus asiaticus and enhanced nutrient efficiency and water absorption [50].

### **Root Inoculation**

The process of root inoculation involves submerging roots in a microbial solution. After being inoculated, the seedling is set up on a growing medium that is appropriate for it. Because inoculation may be done on seedlings of similar sizes, this method allows for the standardization of plant size. Through putting the inoculum in direct contact with the host roots, this inoculation approach also improves root colonization [16]. The various mechanisms employed by microbes to co-operate and compete on root suggest that microbe-microbe interactions play fundamental roles in shaping and structuring microbial networks in environment. The root of most the plant is associated with mycorrhiza. The mutualism with mycorrhizas significantly enhance the active surface of roots, thereby facilitating exploration of a larger soil volume for nutrients and water uptake and also increase the translocation between the roots and shoots of the host plant [51]. A study concluded that induction of induced systemic resistance (ISR) against the fungus Colletotrichum

*graminicola* was reported when *Pseudomonas putida* was applied as a root inoculant in maize plants [52]. In a similar study showed that *Burkholderia* sp. improved *Vitis vinifera* resistance to low temperatures, altered its metabolism of carbohydrates, and enhanced plant growth and yield through root inoculation Fernandez et al. [53].

### Seed Inoculation

Co-culture inoculation treatment with seed is cost-effective and accessible method for field applications [54]. The inoculants are mixed with the seeds manually, with the use of huge dough or cement mixers, inexpensive rotating drums, or mechanical tumbling gadgets. Beneficial microorganisms can inoculate seeds to assist and prevent infections and to colonize the roots of the seeds when they are planted in soil [55]. The primary benefit of using the microbial consortia seed inoculation technique is that it transports bacteria directly to the rhizosphere, where they can bind with plants [55]. When seeds are inoculated in leguminous plants, rhizobia proliferate in the rhizosphere, where they colonize further, form nodules, and fix nitrogen to maximize productivity and yield [56]. Although seed inoculants can interact with fungicides used for seed treatment, they also establish the plant before the pests do and can strengthen microbial defenses. In contrast, mature plants require the suppression of an established microbiome in order to develop an entirely distinct one [57, 58]. There are many drawbacks to seed inoculation. Only a little quantity of inoculant can be coated on each seed, especially small ones. This could be a constraining factor since most PGP microbe may require a threshold of bacteria for successful inoculation. The sowing device has the potential to remove an inoculant that is not securely bonded with pelleting [39]. In a study by Kaur et al. [20] coated barley seed with microbial consortium with sugar solution by 1:1 ratio of before sowing. In a similar study, Negi et al. [19] treated Aegilops kotschyi and wheat seed with microbial mixture and sugar solution in ratio of 1:1 before sowing.

# **Commercialization of Microbial Consortia**

Commercialization of microbial consortium started in 1895 when Nobbe and Hiltner introduced the rhizobia product under the "Nitragin" name. N. V. Joshi initiated the marketing of *Rhizobium* in India to promote the growth of leguminous plants [59]. Throughout its ninth five-year plan, the Ministry of Agriculture launched the National Project on Development, which aimed to popularize and promote the production of biofertilizers while establishing standards for various biofertilizers, training, and exploitation. The National Biofertilizer Development Center was established with six regional centers [60]. Worldwide, there are over 700 products available in the market and more than 200 biopesticide active ingredients are registered. In 2008, there were just 15 biopesticides authorized for use in India under the IA (1968), accounting for a mere 4.2% of the total pesticide market. Nevertheless, growth at a pace of 10% is expected to occur over the next several years [61]. The government measures in favor of sustainable and environmentally friendly agriculture have a significant impact on Asia. In India, there are about 100 public and commercial enterprises that produce biofertilizer; the following is a list of a few of these companies and the important products they produce. The Ministry of Agriculture passed a new decree on the control of biofertilizer production and marketing standards concerning different kinds of microorganisms. The product should fulfill seven quality parameters, like physical form, minimum count of viable cells, contamination level, pH, particle size in the case of carrier-based materials, maximum moisture percent by weight of carrier-based products, and efficiency character [51]. In bacterial bioproducts, the minimum viable cells to be maintained are  $5 \times 10^7$  CFU/g<sup>-1</sup> for solid carrier or  $1 \times 10^8$  CFU mL<sup>-1</sup> for liquid carrier. For products containing mycorrhizal fungi, at least 100 viable propagules must be present per gram of product. Nitrogenfixing efficiency of biofertilizer product should be capable of fixing at least 10 mg N g<sup>-1</sup> of sucrose consumed and for phosphate solubilization product a zone of solubilization of at least 5 mm in a media. AMF products should provide 80 infection points in roots  $g^{-1}$  of inoculum [62].

# Applications of Microbial Consortia in Agriculture

PGP microbe as microbial consortium enhances agronomic efficiency by reducing production costs and environmental pollution. This is achieved because effective PGP microbes decrease the need for chemical fertilizers [63]. Different microbial communities, including fungi, bacteria, actinomycetes, and yeasts, serve as inoculants, primarily promoting plant growth through various mechanisms. These include nitrogen fixation, phosphate, and potassium solubilization, exopolysaccharide secretion, biocontrol activities, organic matter decomposition, and siderophore production [63]. Microbes are employed to release soil nutrients for crops without harming soil fertility, ensuring an environmentally sustainable approach. Prior research has demonstrated their beneficial impacts on the growth and yield of diverse crops, soil types, and even under biotic and abiotic stress conditions. Moreover, these microbes have also proven effective as biocontrol agents against a range of plant pathogens [1]. Microbial consortia have demonstrated their potential as sustainable enhancers of plant growth and as aids in coping with various environmental stresses. Given the extensive, long-term evolutionary interactions between plants and microbes, it is highly likely that there are still many undiscovered benefits that can be harnessed from PGP microbes [2].

PGP microbe are free-living bacteria that play a direct role in promoting plant growth and enhancing root systems in plants [64]. Various pieces of literature have revealed that these bacteria enhance plant growth and crop yields through their plant growth-promoting activities. The literature shows that a microbial consortium has a positive impact on PGP activities. Numerous reports indicate that a wide array of microorganisms thrive in their challenging environments, engaging in interactions with other microorganisms, both intra- and interspecifically. In natural environments, the majority, around 99%, of microorganisms exist in the form of microbial consortia [65]. Numerous studies have demonstrated that individual microorganisms can have a positive impact on plant growth. However, in natural settings, it is evident that multiple species within microbial consortia can perform a broader range of beneficial functions for ecosystems compared to single microorganisms. The interaction between PGP microbes and plants synergistically contributes to the overall benefits for the plant microbiome [66]. Plants further support the growth of PGP microbe by producing various storage substances and root exudates, which serve as sources of nutrition for these beneficial microbes [67]. Microbial consortia are abundant in various natural settings, such as biofilms, food products, soils, and wastewater. These consortia are prevalent in soil and exhibit superior performance compared to individual microorganisms in accomplishing multiple functions. When employed as inoculants, they demonstrate the capacity to thrive in dynamic environments, as they can occupy a wider resource niche within the soil when working together rather than individually. This enables them to compete more effectively with the native soil microorganisms [68]. Furthermore, considering the intricate interactions between soil microbiomes and plants, utilizing microbial consortia appears to be a more practical strategy compared to using single microorganisms as inoculants [69].

In an investigation, microbial mixture of *Bacillus megaterium*, *Arthrobacter chlorophenolicus*, *Enterobacter* sp., and *P. aeruginosa* increased grain yield by 75.80% and 40.09% under greenhouse and natural conditions, respectively [70]. In an another investigation, microbial mixture of *Bacillus cereus*, *Lysobacter antibioticus*, and *Lysobacter capsici* increase the yield by 2909.8 kg/666.67 m<sup>2</sup> [71]. In a finding, microbial consortium of *Enterobacter* spp. ZW32, *Ochrobactrum* sp. SSR, and *Enterobacter* spp. was inoculated on wheat crop. The study revealed that mixture of microbial strain significantly enhance 15% grain yield [72] (Table 1).

### **Limitations and Challenges**

An essential component of any effective co-cultivation system is strain compatibility [73]. In addition to being able to grow effectively in the same growing conditions media, pH, temperature, and oxygen requirement. The cocultivation constituent stains must also be able to avoid producing harmful substances that might seriously impair the other microbial community members [74]. Microbial strains from the same species can be used to meet these requirements because their growth rates and requirements for growing environments are identical [75]. However when several strains from various species are employed to build the synthetic microbial consortium, issues occurs since different species have varied requirements for media and have considerably different growth rates. Successful synthetic microbial consortia not only carry out the desired functions but also sustain cell growth in a stable and robust way. More stable relationships among consortium members are formed when they highly depend on each other. Microbial interactions that lead to the interdependence and stable relationships include cross-feeding, detoxification, and biofilm formation, which are important consortium design principles [76].

It is not intended for the industrial fermentation process used in bioproduction for co-cultivation partners to use the same growth resources as this will lead to unstable co-cultivation and competitive exclusion. Nutritional divergence or syntrophy techniques have been used in co-cultivation systems to overcome this problem. These techniques provide effective energy and carbon channeling, which contribute to the formation of dynamic and symbiotic microbial interactions within the consortium [77]. It is challenging to use this strategy, though, because every organism has different nutritional needs and preferences. Consequently, it is preferable to have cross-feeding or nutritional divergence within a co-cultivation since it enables the removal or reduction of a microbial species from the consortium and permits coexistence [78].

The most significant obstacle to bioproduction in microbial consortia is maintaining the population ratio at the desired level during co-cultivation. The population composition of co-cultivations can vary significantly because of a number of reasons, including substrate competition, variations in doubling times, and hazardous by-products generated by consortium members. As the culture volume increases, the stability of the culture population ratio declines, which could result in system heterogeneity [79]. To prevent one strain from eradicating the other, there are strategies to maintain the strain-to-strain ratios among the co-cultivation members. Although it is frequently seen that the sub-population ratio varies or fluctuates over

2				
S.N	Microbial Consortium	Crop Inoculated Effect	Effect	References
1	Bacillus megaterium + Ensifer adhaerens + Pseudomonas fluores- cens	Wheat	Decreasing electrolyte leakage and increasing chlorophyll contents, relative water contents, and positive impact on shoot length, root length, shoot fresh weight, and root fresh weight	Khan et al. [81]
7	Bacillus sp.+Azospirillum brasilense +Azosprillum lipoferum	Wheat	Decreases electrolyte leakage and enhanced the chlorophyll content, RWC, proline content, amino acid, and antioxidant enzymes	Akhtar et al. [82]
ŝ	Pseudomonas aeruginosa+Trichoderma harzianum+Bacillus subtilis	Pea	Increase in plant length, total biomass, number of leaves, nodules and secondary roots, total chlorophyll, and carotenoid content	Jain et al. [83]
4	Pseudomonas extremorientalis+Bacillus subtilis+Bacillus amyloliquefaciens	Pearl millet	Increase length and biomass of root/shoot, chlorophyll, carotenoids, total soluble sugar content, phenolics, and flavonoids	Kaur et al. [28]
S	Siccibacter colletis + Enterobacter huaxiensis + Pantoea sp.	Faba bean	Increase root and shoot dried biomasses and chlorophyll content, P uptake	Chamkhi et al. [84]
9	Rahnella sp.+Bacillus tropicus+B. megaterium	Wheat	Enhance growth as well as physiological parameter including shoot/ root length, fresh/dry weight and chlorophyll, carotenoids, total soluble sugar content, phenolic and flavonoids content	Negi et al. [19]
٢	Erwinia sp. + Chryseobacterium arthrosphaerae + Pseudomonas gessardii	Barley	Increase root/shoot length and biomass, chlorophyll, carotenoids, phenolics, flavonoids, and soluble sugar content	Kaur et al. [20]
8	Erwinia persicina + Halomonas aquamarina + Pseudomonas extremorientalis	Chilli	Increased shoot/root biomass and length; number of leaves, branches, and fruits per plant and content of chlorophyll, total soluble sugar, phenolics, and flavonoids	Devi et al. [33]
6	Acinetobacter guillouiae + Acinetobacter calcoaceticus	Onion	Positively impacted shoot length, root length, biomass, phenolic, flavanoids, total soluble sugars, and chlorophyll content	Kour et al. [21]
10	Stenotrophomonas rhizophila + Pseudomonas marginalis + Bacil- lus sp.	Foxtail millet	Improved the growth and physiological parameters	Kaur et al. [34]
11	Azotobacter sp. + $Pseudomonas$ sp.	Malt Barley	Highest and significant effect on grain yield, harvest index, biological yield, plant height, and thousand seed weight	Tadesse, Melak [85]
12	Pseudomonas gessardii + Erwinia rhapontici	Rajgira	Enhance shoot/root length and biomass and chlorophyll content, total sugar content, phenolics, and flavonoids content	Devi et al. [27]
13	Bacillus thuringiensis+Bacillus horikoshii+Pseudomonas trivialis	Sweet pepper	Increase plant length, root length, fresh weight, and biomass of the plant and chlorophyll, carotenoids, flavonoids, phenolics, and total soluble sugar content	Devi et al. [86]
14	Pseudomonas aeruginosa+Trichoderma harzianum+Bacillus subtilis	Pea	Increase in plant length, total biomass, number of leaves, nodules and secondary roots, total chlorophyll and carotenoid content, and yield	Jain et al. [83]
15	Azospirillum + Azotobacter chroococcum + Bacillus megaterium + Pseudomonas fluorescens	Ashwagandha	Significantly increased plant height, root length, and alkaloid content	Rajasekar, Elango [87]
16	Azotobacter chroococcum + Priestia megaterium + Pseudomonas sp.	Pigeon pea	Significant increase in plant attributes such as shoot length, root length, fresh weight, and dry weight	Srivastava, Sharma [88]
17	Bacillus licheniformis + Bacillus sp. + Pseudomonas aerugi- nosa + Streptomyces fradiae	Sunflower	Increased the plant growth and yield and reduced the SNV disease incidence	Srinivasan, Mathivanan [89]

 Table 1
 Effect of microbial consortium on different crops

S.N	S.N Microbial Consortium	Crop Inoculated Effect	Effect	References
18	18 Bacillus sp. + Delftia sp. + Enterobacter sp. + Achromobacter sp.	Tomato	Increase in leaf, shoot, root dry weight, leaf number, shoot length, root length, secondary roots, and chlorophyll content	Kapadia et al. [90]
19	Azospirillum brasilense + Gluconacetobacter diazotrophi- cus+Herbaspirillum seropedicae + Burkholderia ambifaria	Onion	Increased plant height, total chlorophylls, crop yields, and bulb dry Pellegrini et al. [91] matter	Pellegrini et al. [91]
20	20 Bacillus megaterium + Arthrobacter chlorophenolicus + Enterobac- ter sp. + Pseudomonas aeruginosa	Wheat	Showed significant increase in plant height, grain yield, and straw yield	Kumar et al. [70]
21	Ensifer meliloti + Rhizobium leguminosarum	Fenugreek	Increased grain yield, maximum increments in vigor index, nodule number, and root and shoot biomass	Kumar et al. [92]
22	Bradyrhizobium sp.+Pseudomonas sp.+Ochrobactrum cytisi	Lupines	Increases plant yield and nitrogen, and decreases plant metal accu- mulation	Dary et al. [93]
23	23 Bacillus paralicheniformis+Bacillus subtilis+Bacillus megate- rium+Bacillus cabrialesii	Wheat	Significant increase in the length of the aerial part of the plant, root Molina-Romero et al. [94] length, total length, stem diameter, circumference, dry weight of the aerial part of the plant, and the biovolume index	Molina-Romero et al. [94]

Table 1 (continued)

the cultivation period, fine-tuning the inoculation ratio between co-cultivation partners has a significant impact on overall productivity. Additionally, research on mutualistic growth has been done to keep the engineered cocultivations' intended composition of the population [80].

# Conclusions

A new approach to alteration in metabolic pathway balancing is provided by microbial biosynthesis via co-cultivation engineering. It increases the range of options for fine-tuning intricate metabolic pathways and can be tailored for effective synthesis of various bioproducts. Compared to mono-cultivation systems, co-cultivation engineering offers a number of benefits, including increased productivity, robustness, modularity, and tolerance (toxic intermediates and waste created by one partner are consumed or degraded by the other partner). Co-cultivation fermentations have the potential to improve production efficiency and enable the use of less expensive substrates. Furthermore, without sacrificing output or quality, artificial consortiums provide a solution to solve the problems associated with the functional expression of complicated biosynthetic pathway enzymes. They may also lessen the work involved in reconstructing recombinant biosynthesis pathways. Co-cultivation of several populations is more difficult since it is unknown how individual strains would behave when grown together using standard techniques, and it may also be harder to regulate as the number of constituent strains or species increases. But recent advances in co-cultivation engineering have significantly increased our comprehension of how microbes interact in communities. Synthetic biology techniques must be used to overcome certain inherent obstacles, even though the potential of microbial consortia seems extremely promising. In the near future, it is expected that co-cultivations, or polycultures made up of several specialized members will be created and used to address the demand for increasingly complex biochemical pathways. The creation of novel microbial consortia with distinct roles should be explored through the use of metabolic engineering and synthetic biology techniques. Since functional genomics (metabolomics, proteomics, and transcriptomics) is still expensive, new methods must be developed to investigate community profiles and interactions among microbes in consortium culture.

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### Declarations

Conflict of interest There are no conflicts of interest to declare.

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