Engineering the Plant Microenvironment to Facilitate Plant Growth Promoting Microbe Association

Augustine T. Zvinavashe[1], Ilham Mardad[2], Manal Mhada[2], Lamfeddal Kouisni[2,3], Benedetto Marelli[1,*]

[1] Department of Civil and Environmental Engineering, Massachusetts Institute of Technology, 77 Massachusetts Avenue, Cambridge, 02139, MA. United States.

[2] Mohammed VI Polytechnic University (UM6P), AgroBioSciences, 43150, Ben Guerir, Morocco.

[3] African Sustainable Agriculture Research Institute-Mohammed VI Polytechnic University (ASARI-UM6P), Laayoune, Morocco.

[*] Correspondence should be addressed to bmarelli@mit.edu

Keywords: fertilizer; biomaterials, rhizobacteria; endophytes; seed coating; inoculation

Abstract

New technologies that enhance soil biodiversity and minimize the use of scarce resources while boosting crop production are highly sought to mitigate the increasing threats that climate change, population growth, and desertification pose on the food infrastructure. In particular, solutions based on plant growth promoting bacteria (PGPBs) bring merits of self-replication, low environmental impact, protection from biotic and abiotic stressors and reduction of inputs such as fertilizers. However, challenges in facilitating PGPBs delivery in the soil still persist and include survival to desiccation, precise delivery, programmable resuscitation, competition with the indigenous rhizosphere and soil structure. These factors play a critical role in microbial root association and development of a beneficial plant microbiome. Engineering the seed microenvironment with protein and polysaccharides is one proposed way to deliver PGPBs precisely and effectively in the seed spermosphere. In this review, we will cover new advancements in the precise and scalable delivery of microbial inoculants, also highlighting the latest development of multi-functional rhizobacteria solutions that have beneficial impact not only on legumes but also on cereals. To conclude, we will discuss the role that legislators and policymakers play in promoting the adoption of new technologies that can enhance the sustainability of crop production.

1. Introduction

Population growth, climate change, desertification and salinization of the earth soils have led to the necessity to build resilient food systems while increasing agricultural output.[1–4] Chemically-derived synthetic fertilizers and pesticides have been used for decades to boost plant growth.[5,6] It is well known that plants primarily require nitrogen, phosphorus and potassium (NPK), for their nutrition. However, these nutrients tend to be the limiting resource in plant growth, thus decreasing the yields.[7] Synthetic fertilizers are responsible for 40 to 60% of the world's food production and are primarily constituted of NPK. Stewart et al [8] reviewed data representing 362 seasons of crop production and reported that a minimum of 30 to 50% of the crop yields can be attributed to synthetic fertilizer use, highlighting the major importance of fertilizer to humanity.[9] Nitrogen based fertilizer production accounts for about 1% of the world's energy consumption while emitting about 1.2% of the global anthropogenic CO_2 emissions that reinforce climate change effects[10,11]. In addition poor fertilizer usage and runoff lead not only to degradation and salinization of soils, but also to eutrophication of our water sources.[11–14] Therefore, upscaling new means to ensure environmentally friendly and sustainable solutions for soil management and agricultural production is required.[15] Furthermore, phosphate is a non-renewable resource[16]. Morocco hosts by far the largest reserve, holding 80% of global rock phosphate[16]. This makes supply a conceivable problem as China, USA and India (the largest food demanders) will runout of phosphate by 2040.[17] Microbes have the potential to increase phosphorus plant intake as most phosphate is held in inorganic insoluble form [e.g., $Ca_3(PO_4)^2$] and organic insoluble/soluble form (e.g., phytate and nucleic acid) which microbes can make available to plants and therefore limit the synthetic phosphorus fertilizer application.[18] The exploitation of microbes has proven to provide environmentally friendly and sustainable solutions that should be pursued, yet it shows some constraints.[14,19]

Chemical fertilizer attributes such as quick and nonspecific action, low-cost production and ease of storage made them widely acceptable.[20] However, their detrimental effects to soils, plants and animals when they are not used efficiently motivate us to find complementary alternatives to optimize their use and, thereby, lowering their impact on soil fertility and biodiversity.[21–23] Further, pests' resistance and high concentration used/overuse are an unresolved problems that generate an increasing demand for sustainable solutions. Therefore, there is a growing interest in the use of microbial fertilizers as complements to synthetic fertilizers and agrochemicals.[24] Nitrogen and phosphorus are the two most important nutrients to plants and applied nutrients in agriculture. Therefore, to secure food supply and farm sustainability, microbial alternatives are necessary to optimize their use. Nitrogen fixing and phosphate solubilizing microbes can be used in co-inoculations (individually or as consortiums) which result in greater plant growth promotion by providing these essential macronutrients while lowering our carbon footprint.

Naturally derived nutrients and soil stressor alleviators have existed for centuries for integrated nutrient and disease management and soil biodiversity for rhizobia and now, they are used for other plant growth promoting microbes.[25] Initially, farmers knew that the soil taken from previous legume-sown field to non-legume field often improved the yield.

The soil transfer approach was followed till the end of the nineteenth century for legume seed inoculation.[26] Advances in the understanding of plant-microorganisms interactions are now well-known and have led to the discovery and exploitation of plant growth promoting microorganisms (PGPMs), which include archaea, bacteria and fungi. However, some can be a biohazard.[27] Plant microbes provide the nutrients that plants require and regulate plant growth. PGPMs facilitate this directly through nitrogen fixation, phosphate solubilization and phytohormone production[28] **(Figure 1)**, and indirectly by preventing the negative effects of phytopathogenic organisms through the production of antimicrobial compounds or the elicitation of induced systemic resistance.[29] PGPMs pertain to the following classes: the rhizospheric microbes found around the soil in the plants rhizhosphere (root system), phyllosphere (aerial parts of plants), rhizoplane (root surface) and endophytes found inside the plants root, stem and leaf system.[30] Implementing solutions that can be used in agricultural practices is crucial. Our focus in this review will be on bacteria given that archaea are still an under-detected and scarcely studied part of the plant microbiome while fungi (which are eukaryotic) are only able to obtain fixed nitrogen through symbiotic interactions with nitrogen-fixing prokaryotes and we believe cannot fix nitrogen. Nevertheless, a recent study showed potential for nitrogen fixation in the fungus-growing termite gut.[31–33]

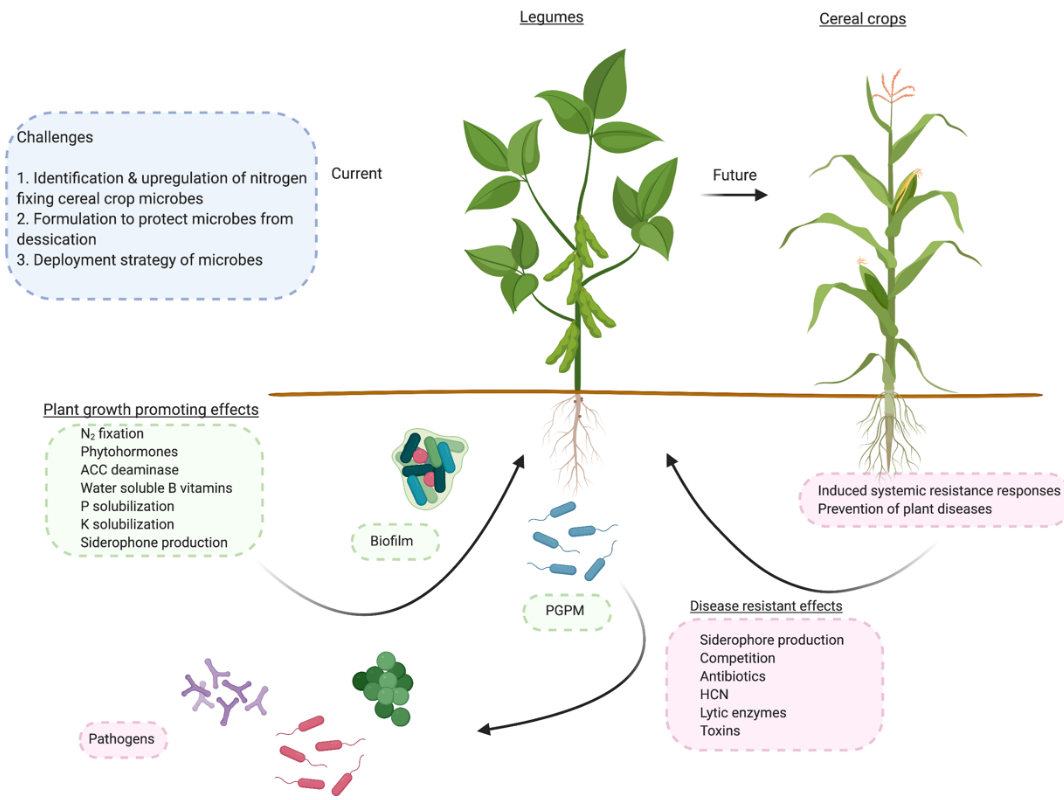

Figure 1. Mechanism of plant growth promoting microbes.

Emerging technologies such as proteomics, metabolomics, transcriptomics and next-generation sequencing and data science has made and will make the discovery of useful compounds, microbe interaction understanding and identification and characterization of microbial inoculants fast and easier.[27] Microbes are very specific to the plant and use case. Therefore, the gathering of data on microbial interactions and learning from this data is essential in the use and delivery of plant microbes. Furthermore, the interplay of microbes in a consortium needs to be better understood as some have synergistic effects as singular strains but may have detrimental or beneficial effects when used in a consortium. The inoculation of plants with a microbial consortium provides better benefits to a plant than with a single isolate.[34,35] This could be because microbial consortia may have synergistic interactions to provide nutrients, remove inhibitory products and trigger each other through biochemical and physical activities that might enhance beneficial effects on plant physiology.[36] Recently, a large-scale genomic comparison of PGPMs discovered that the dominant bacteria associated with plants are Actinobacteria, Bacteroidetes, Firmicutes, and Proteobacteria, which had also been suggested in previous studies.[37,38] Microbiologists are working on better understanding microbial communities and this will be essential in understanding how to deliver microbes in different soils that possess different microbial communities and nutrients. It was suggested that inoculated bacteria are actively influenced by the plant genotype, cropping conditions and by co-inoculated or residing bacterial populations which can considerably influence the resulting PGPB-effects.[39,40]

Microbes can be classified as either gram negative or gram positive. Gram positive bacteria possess a thick (20-80 nm) cell wall as outer shell of the cell. In contrast gram negative bacteria have a relatively thin (<10nm) layer of cell wall, but harbor an additional outer membrane with several pores and appendices.[41] The relatively thin cell wall makes gram negative microbes delicate to dry, handle, resuscitate and deliver. Currently, there are several means to deliver microbes in the soil but they are not efficient and lack ease of implementation in remote regions of the world, where agriculture practices cannot account for handling of living bacteria.

Plant growth promoting bacteria (PGPBs) are endophytic or rhizospheric and are known to associate with a variety of crops in plant root structures, leaves and surrounding soils.[42] In an effort to better understand the microbial delivery tools that are currently used to deliver PGPBs effectively, it is first necessary to take into account the best strain of microbe or a microbial consortium for the intended effect on the target crop. Then, the formulation of the inoculant should be addressed and, finally, the delivery method (**Figure 2**).[43] Currently, delivery happens through biopriming, which is a biological process of seed treatment that mixes seed hydration and seed inoculation with plant beneficial microorganisms in order to improve seed's germination and their protection against soil borne pathogens, achieving seedling and vegetative growth.[44] However, given it is labor intensive nature, this process is mostly appropriate for low-medium volumes of high value crops.[45] Soil inoculation is also used as an alternative. However, it requires high volumes of inoculant and is labor intensive thus expensive and may be restricted by local environmental regulation and health concerns.[46] Seed coating has the potential to be a cost-competitive and time-saving approach for crop production and protection. Nonetheless, microbial seed coating is hindered by low performance and standardization, which limit its broader use.[46]

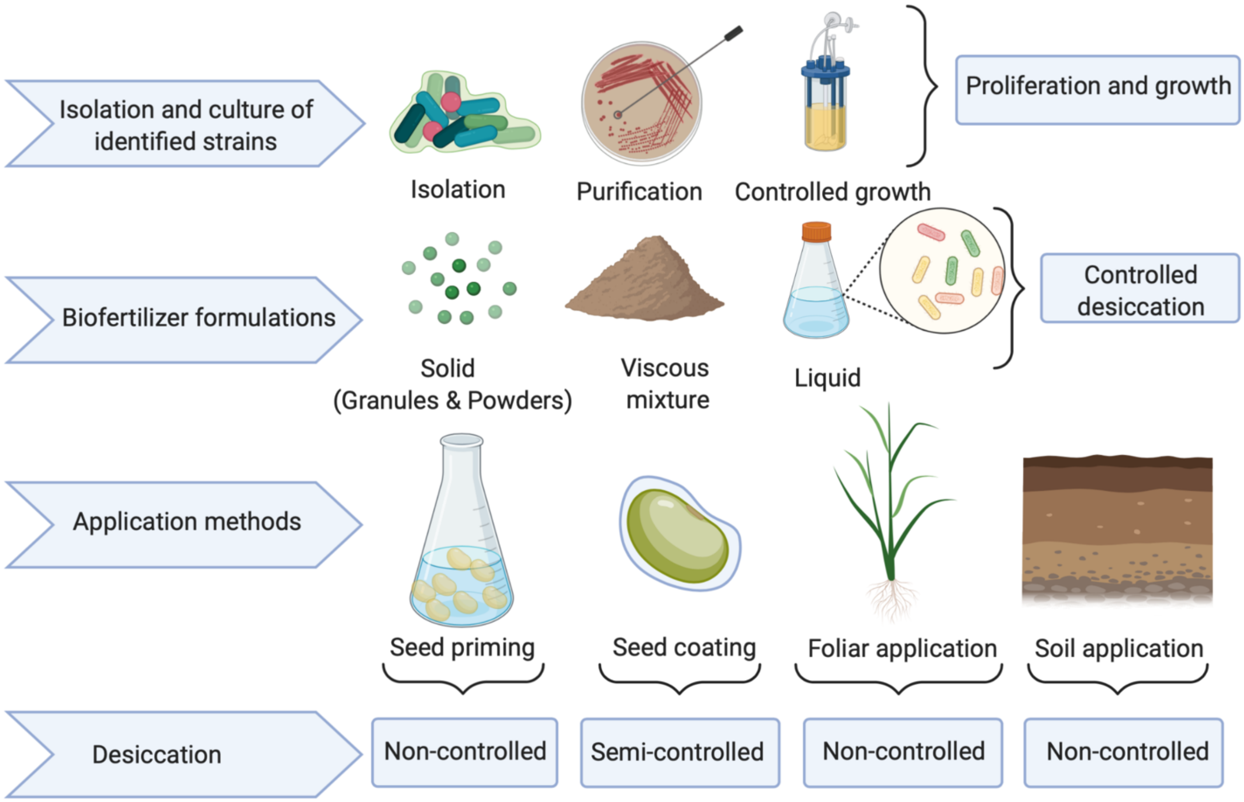

Figure 2. From identification to formulation and application of microbial fertilizers. Application procedure and formulation control the desiccation process.

2. Challenges

Several challenges such as unpredictability of results, difficulties in the identification and isolation of bacterial strains in field experiments, poor understanding of specific mechanisms that regulate the interplay between microorganisms, plants and soil have limited the use and effectiveness of PGPBs.[47] In this context, two key aspects that dominate the effectiveness of inoculation are the microbial isolation and the application technologies.[43] The design and delivery of microbial consortia through inoculation is challenging and requires the understanding of their modes of interaction, microbial adhesion to seeds, plant root colonization and antagonistic relationship interactions, if present.[48] Differences in root communities have been attributed to plant host effects and microbial host preferences, as well as to factors pertaining to soil conditions, microbial biogeography and the presence of viable microbial propagules.[49] The unprotected, inoculated bacteria must compete with the often better-adapted native microflora and withstand predation by soil microfauna.[43] The environmental conditions also affect the inoculant efficacy and adverse abiotic stresses (hot, dry and saline conditions) can cause rapid decrease in PGPBs populations.[50,51] The following challenges are important in improving PGPBs performance:

Desiccation

Microbial desiccation affects viability of microorganisms. The number of metabolically or physically active microbes is the leading factor towards the efficacy of PGPBs when applied to the seed surface.[52] Desiccation is the process of water removal from (or extreme drying of) an organism, therefore drought stress affects microbial biodiversity in soils. Microbial viability is important as it increases the effectiveness of microbe infection, permitting PGPBs to induce a positive effect in plants. Therefore, desiccation tolerant microbes are highly desirable because they can remain in soils and inoculant formulations for a longer time than those that are not desiccation tolerant.[34] A recent study reported that 95% of PGPBs does not survive in the time intercurring between inoculation of the seed and planting (considering a 4 hour time window) and that 83% of the surviving microorganisms dyes in soil within 22 hrs.[53] In nature, there are anhydrobiotic organisms that are able to survive desiccation by going into a dormant state in which metabolism is undetected. Once rehydrated, they are able to restore their metabolic processes.

Learning anhydrobiosis from such organisms will be a beneficial approach in finding ways to mitigate desiccation stress. Some PGPBs have acquired desiccation tolerant mechanisms such as the production of intrinsic trehalose.[53] The trehalose produced may regulate most of the plant's enzymatic and non-enzymatic responses by supporting the production of the plant's collection of phytohormones.[54] Other organisms, called xero-halophiles, are extremophiles and live in areas where soil is very saline and dry. Desiccation is a topical subject in microbial fertilizers because the efficacy of microbe fertilizer is correlated with viability of the microbes. As the agriculture field looks for opportunities to transition from synthetic fertilizers to microbial ones (also known as biofertilizers), there is an increasing interest in scalable technologies that address desiccation tolerance by providing, for example, a microenvironment that facilitates microbe survival and growth in the form of seed coatings that then degrade in the soil and deliver PGPBs. Alternative technologies to boost PGPBs performance include the selection of desiccation resistant strains, and the use of synthetic biology tools to provide desiccation resistant genes.

Climate Change

Climate change has impacted soil microbial communities resulting in increased atmospheric CO_2 concentration, temperature, precipitation and drought.[55] The effects have been both positive and negative. Numerous studies have showed how elevated CO_2 levels increased the abundance of arbuscular and ectomycorrhizal fungi, whereas the effect on PGPBs and endophytic fungi were more variable. Mostly, PGPBs were beneficial under elevated CO_2,[55] which leads to higher carbon availability in the rhizosphere and may alter root exudation composition. Root exudates play a huge role in the structure and function of microbial communities. This indicates that colonization of plants depends on compounds produced by plants, which are affected by climate change factors such as temperature and drought. In these conditions, different microorganisms show potential for different functional activities that leads to altered community structures and may be used to impart different colonization strategies by inoculating microorganisms such as arbuscular mycorrhizal fungi to change the composition of the microbial community.[56] Further, at elevated CO_2 concentrations, nitrogen becomes a growth-limiting nutrient and as such nitrogen fixing and acquiring microorganisms may gain increasing importance.

Temperature effects are coupled with soil moisture, thus difficult to deduce. Soil microorganisms and the processes they mediate are temperature sensitive. Decomposition of organic soil matter, soil respiration, and growth of microbial biomass increases with temperature. It has been hypothesized that temperature effects are transient; as temperature increases, the soil carbon substrates are quickly depleted by enhanced microbial activity and because of tradeoffs microbial communities either adjust, shift in composition, or constrain their biomass to respond to altered conditions and substrate availability.[57,58]

Drought leads to soil moisture stress, which impacts the soil microbial community, however it is less investigated than CO_2 or temperature. Drought amplifies the differential temperature sensitivity of fungi and bacteria.[55] Small changes in soil moisture can shift fungal communities from one dominant member to another while bacteria remain constant. Typically, drought reduces fungal colonization, although the outcome can be strain dependent.

Soil pH

Soil pH is one of the most influential factors affecting the soil microbial community.[59] pH greatly affects abiotic factors, such as carbon availability, nutrient availability, and the solubility of metal ions. Furthermore, pH may affect biotic factors, such as biomass composition of fungi and bacteria in both forest and agriculture.[59] The challenge of studying pH effects are its varied effects on multiple factors. Rousk et al showed that as pH drops from 8.3 to pH 4.5, a fivefold decrease in bacterial growth and fivefold increase in fungal growth was measured. Fungi generally exhibit wider pH tolerance when compared to bacteria, which tend to tolerate narrower ranges.[60] The shift in fungal and bacterial importance as pH drops has a direct negative effect on the total carbon mineralization. Below pH 4.5, there is general microbial inhibition, probably due to release of free aluminum and the decrease in plant productivity. Conversely, studies conducted from soils from North and South America have shown that both the relative abundance and diversity of bacteria increased with soil pH, considering ranges between pH 4 and 8.[60] The relative abundance of fungi was, however, unaffected by pH and fungal diversity was weakly positively related.[60]

Competition in the Soil and Microbe Concentration

Inoculated legume root nodules are mostly formed by indigenous microbes present in the soil.[52] Microbe competition is one of the key determining factors for infection effectiveness. Rhizospheric microorganisms connect plants and soils and together develop an ecosystem that provides nutrient life cycle and soil fertility.[61] Technological advances in DNA sequencing, molecular ecology and data science have provided the tools to study plant-associated and soil microbial diversity and to assess the implication of this diversity on ecosystem functioning.[62] When microorganisms are delivered into the soil, we need to consider the surrounding ecosystem that will be in competition with them. The viability, concentration and delivery method of microbes become vital as a competitive advantage over other microbes as the physiological state of microbes can prevent biomass buildup. Therefore, microbe release mechanism in soil becomes paramount as it affects the concentration and location of delivery that are impacted by rhizospheric microbe competition. A threshold number of cells, which differs among species, is essential to obtain the intended positive plant response. For example, it has been reported that 10^6–10^7 cells·plant^{-1} are necessary for the PGPB Azospirillum brasilense.[63] Oliveira et al, showed that a consortium of microbes improved plant growth more than a singular isolate inoculation.[48] Gottel *et al*. and Shakya *et al*. found that the ecological niche (endosphere vs. root) outperformed other measured factors (soil properties, season, plant genotype, etc) (upland vs. lowland) in shaping microbial communities.[49,64]

Soil Structure

Soil structure is the arrangement of primary soil particles and the pore spaces between them. Microbe-plant interactions are influenced by the soil type, soils that share a certain set of well-defined properties.[49] Biological linkages between soils, roots and the atmosphere are poorly characterized. However, Bonito et al showed that bacterial communities in the root are more tightly structured by plant host species than by soil origin.[49] Plants, soils and microbiota interact and function in a zone known as the root microbiome,[65] which is characterized by elevated rates of respiration, nutrient turnover, and carbon sequestration, highlighting its importance to the functioning of terrestrial ecosystems.[66] The nutrient concentration, pH and water content play an active role on microbe colonization. Microbes are very specific therefore have differing niche microenvironments that accommodate them best. The distribution of bacterial and fungal communities and their function varies between different aggregate size classes.[67] Further, compaction of soil has detrimental effects as it affects physical properties of soil such as bulk density, soil strength and porosity. Compaction limits the mobility of nutrients, water and air infiltration and root penetration in soil.[68] Juyal et al. have shown how increasing soil bulk density (compaction) significantly reduced the number of microorganisms in soil and their growth rate. Good soil structure provides an array of niches, such as substrate availability and redox potential, which can house diverse microbial communities.[69] Microbes reside in pores and inner surfaces of aggregates as microcolonies of 2–16 microbes each, and extensive colonization is restricted to microsites with higher carbon availability, e.g., rhizosphere and outer surfaces of freshly formed macroaggregates.[70] Location of aggregates in relation to roots, organic residues, and macropores is more important for determining the microbial community composition and their activity.[69] Understanding the microbes niche environment will help build predictive models and skill us in shaping the rhizosphere of the plant as microbes are very specific with regards to conditions required for colonization.

Perspective

PGPBs are plant and soil specific, which makes them challenging to deploy universally. However, as our understanding of soil structure, soil pH, impact of climate change, soil microbe concentration and desiccation impact plant and soil microbe interaction increases, the efficacy of microbe-based fertilizer can be enhanced by precise microbe selection, developing models based on plant, and investigating microbe and soil interactions. All the extrinsic factors influencing PGPBs growth and metabolism are coupled together and understanding how they all interact will be key to design highly effective techniques to develop and deploy, at scale, biofertilizers.

3. Formulations

Rhizobia bioformulations have been on the market for centuries in numerous forms. Commercial biofertilizers can be solid carrier based (organic or inorganic), liquid formulations, synthetic polymer based or metabolite based formulations.[51] The formulation is composed of the microbe, carrier material, and additives. The first commercial nitrogen biofertilizer of rhizobia, ‘Nitragin’ was patented by Nobbe and Hiltner.[51] Initially, inoculation procedure entailed transferring soil from legume grown soils to soils that will host plants. Following this first technology, solid based carriers came into use in the early 1900’s. Even today, many of the microbial inoculants all over the world are based on solid based carriers, mostly peat formulations. This has been true for well-developed legume inoculants based on selected rhizobial strains, due to peat bacterial protection properties,[71] such as high water holding capacity, chemical and physical evenness, non-toxic and environmentally friendly nature.[72] However, peat is very inconsistent and is a non-renewable resource making it unusable on a large scale.[73] Thus, interest in substitutes grew and alternatives such as lignite, filter mud, coal-bentonite, cellulose, coal, soil, charcoal, manure, compost, powdered coconut shells, ground teak leaves and wheat straw have been used as solid carrier materials.[51] Granular carriers were also developed for direct application to the soil, which made handling, storage and application easier.

Liquid formulations were developed as alternatives to solid carriers due to their limitations such as environmental impact and carbon emissions of peat-made solid carries.[72] Further, liquid formulations are better suited for mechanical sowing in large fields.[43] In 1958, freeze-dried inocula came on to the market, then gel based microbial inoculants that entrapped rhizobia in polymer gels such as polyacrylamide-entrapped *Rhizobium* (PER), alginate-entrapped *Rhizobium* (AER), and xanthan-entrapped *Rhizobium* (XER); which gave satisfactory results in wet conditions.[51,74] In the early 2000’s, the modification of liquid formulations by addition of additives and cell protectants were proposed. The additives promote cell survival in storage and after application to seed or soil.[75] Commonly used additives for rhizobial inoculants were polyvinyl pyrrolidone (PVP), carboxymethyl cellulose (CMC), gum arabic, sodium alginate and glycerol.[51] PVP protects microbes from desiccation and harmful seed exudates and CMC's rheological property increases the gel viscosity of carriers to make it more suitable for viability of rhizobial cells.[51] Further, genetic modification of rhizobia is being developed to improve the efficacy of nitrogen fixation in new formulations, such as upregulating nitrogen fixation.[76] The emerging technique of secondary metabolites addition (flavonoids and phytohormones) to bioformulations increases agricultural productivity by improving the inoculants efficiency.[77] The addition of flavonoids to rhizobial formulations during growth, significantly alleviates the effects of adverse conditions,[78] enhances nitrogen fixation [79], improves the rhizobial competitiveness and nodulation.[51] The cost associated with flavonoids isolation or synthesis is sometimes justified by the low concentrations used in the final formulation.[80,81]

Despite, the abovementioned technologies, bioformulations still face many limitations. Inoculation formulations have improved microbial survival during storage of products, but these efforts have not improved survival on the seed or in soil.[52] Bacterial survival on the seed are mainly affected by three factors: desiccation, the toxic nature of seed coat exudates and high temperatures.[82] Therefore, there is a need to find biomaterials that could provide a microenvironment to protect microbes from desiccation while also having the mechanical properties to conform around a seed (**Figure 3**).[83] Biomaterials are biocompatible, biodegradable and abundant, thus have potential in enhancing food security and safety. [84–87]

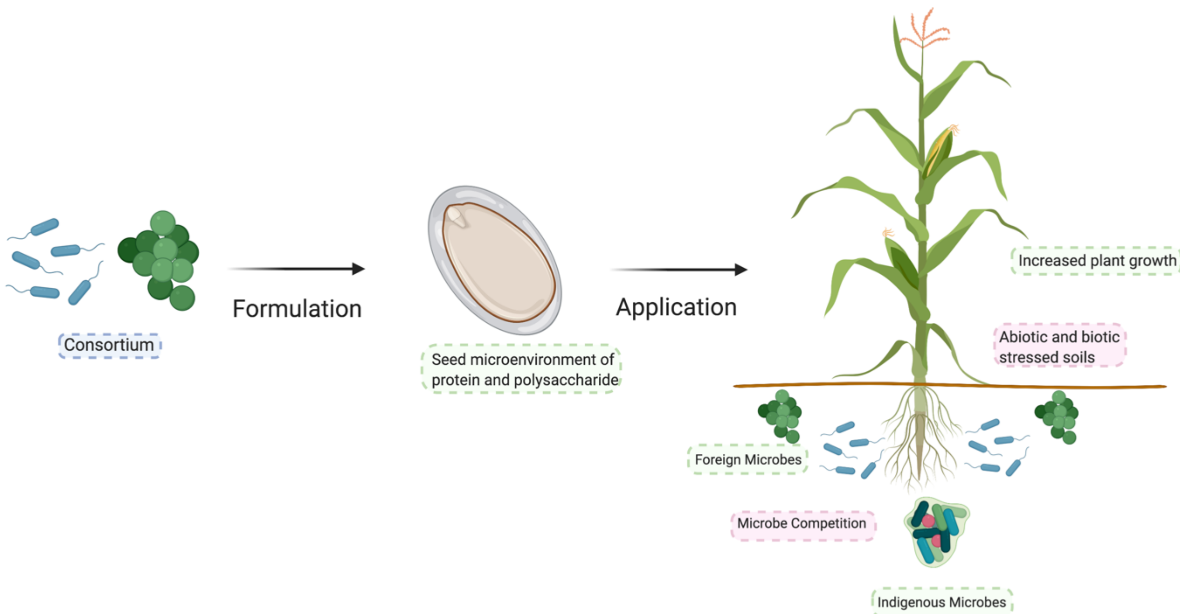

Figure 3. Seed coating technology encapsulates and protects microbes while providing a targeted in situ release of payload to be delivered.

Efficacy of formulations depends on their shelf life, which depends on several factors such as production technology, carrier and packing material used, transport activity and farmers' practices to sustain the quality of inoculants.[88] Factors related to production processes (quality and marketing standards) are also important for consistency and user uptake. Currently, the storage, preparation and application of formulations needs special facilities and skills, which most farmers and suppliers do not possess.[89] Therefore, an easy to use alternative is necessary for better adoption. The current problems with most formulations are a lack of robust scientific data. According to Brockwell et al [90], 90% of inoculants have no impact on target crop. Further, Herrmann et al.[91] reported that more than 50% of the inoculants have high levels of contamination. Contaminants have detrimental effects on the quality of rhizobial inoculants and 25% contaminants of the commercial inoculants can be opportunistic human pathogens. Therefore, many inoculants produced globally, because of lack of quality control, tend not to perform well. Thus, there is a requirement for strict regulations for rhizobial bioformulations to overcome the abovementioned problems related to worldwide production and application of biofertilizers. In the future, emphases should be given to techniques that increase population density and survival of rhizobial strains in inoculants and minimize operator exposure to high dose of PGBPs whether in solution or in water droplets. Additionally, survival of cells is mandatory for better commercialization of rhizobial inoculants in the global market.[92]

Nano-bioformulations of biofertilizers has emerged as one of the most promising techniques to achieve this goal. It comprises nanoparticles made up of organic or inorganic materials, that interact with microorganisms and enhance their survival by providing protection from desiccation, heat, and UV inactivation. Applications of nano-bioformulations also include environmental cleanup strategies.[93] In 2015, PGPBs such as (*Pseudomonas fluorescens*, *B. subtilis* and *Paenibacillus elgii*) treated with silver, aluminium, and gold nanoparticles have been shown to support plant growth and increase pathogen resistance.[94] The release of such nanoencapsulated biofertilizers into target cells is operated in a very controlled manner, free from any harmful effects and increasing the adhesion of beneficial bacteria within the root rhizosphere.[95] Additionally, nanobiofertilizers may be considered as an alternative to chemical pesticides,[96] although the deployment of nanoparticles in the environment needs to satisfy stringent requirements imposed by policymakers.

The application of phyto-nanotechnology on agriculture could change the traditional plant production systems, providing the controlled release of agrochemicals (e.g., pesticides, herbicides, fertilizers) and target-specific transport of biomolecules (e.g., activators, nucleotides, proteins). Nanoencapsulation using biodegradable materials also makes the assembled active elements straightforward and safe to be handled by the farmers. Advanced understanding of the interactions between nanoparticles and plant responses (uptake, localization, and activity) could transform crop production through improved disease resistance, nutrient use, and crop yield.[97]

The use of polymeric inoculants and alginate beads have already been tested and need more exploration for their future use.[43,51] Furthermore, the use of stress tolerating microbes/rhizobia in inoculations is also thought to be imperative in developing bioformulations that will survive in stress conditions (high temperature, drought, salinity).[98,99]

The use of genetically improved rhizobia as inoculants has some legislative constraints because it requires permission from environmental protection agencies to release into the environment and due to the little understanding of microbial ecology.[100] Further, the majority of microbial seed inoculation involves private companies (agrichemical and seed companies) that rarely disclose their data and formulations[45], although there is a compelling need to develop a more comprehensive knowledge that integrates academic efforts to speed up advancements and the development of disruptive technologies.

Perspective

Peat-based formulations have been traditionally used for the delivery of microbe-based fertilizers. These tend to be good at providing the niche for microbe growth when outside the soil and when inoculated. However, since peat is a non-renewable resource, new formulations are required. Liquid-based formulations have been developed, however performance in microbe preservation can be improved to ensure high efficacy of the inoculant. As we learn new lessons on how microorganisms survive desiccation, e.g. by looking at tardigrades production of trehalose and intrinsically disorder proteins to promote water substitution and vitrification, new strategies can be designed to engineer formulations that better protect and store microbes outside the cold chain and in operational conditions before deployment in the field.

4. Rhizosphere and Endosphere

Rhizobacteria

The rhizosphere is the region of soil directly surrounding the root system that is directly influenced by root secretions and associated soil microorganisms known as the root microbiome.[101,102] Rhizobacteria implies a group of bacteria found in the rhizosphere that can colonize the root system.[103] It has been demonstrated that bacterial cells first colonize the rhizosphere following soil inoculation.[104] Therefore, microorganisms delivered in the soil need to be able to colonize the rhizosphere before they can have an impact on plant health and metabolism. Bacterial cells have been visualized as single cells attached to the root surfaces, and subsequently as doublets on the rhizodermis, forming a string of bacteria.[105] Colonization then occurs on the whole surface of the rhizodermal cells.[106] For microbes to produce plant growth promoting factors, they need to be able to colonize the rhizosphere and/or the rhizoplane during an extended period characterized by strong microbial competition with rhizosphere competent microbes (microorganisms that have the capacity to effectively build a population of microorganisms on plant roots or in the vicinity).[107] Furthermore, root colonization is complex and non-uniform. This can be explained by different factors such as varying root exudation patterns released by plants and containing chemoattractant to promote microbe colonization and growth.[108] Rhizosphere colonization is however a complex system influenced both by microorganisms competition during inoculation and rhizosphere competence of the microbe. We are yet to fully understand these interactions, which are soil specific as a microbe needs a specific niche to perform optimally.

Endophytes

There are types of microorganisms that do not only colonize the rhizosphere but also enter and colonize plant tissue for beneficial effects, i.e. endophytes.[105] Studies have shown how plants host a diverse group of endophytic microbes and most endophytes are derived from the rhizosphere, e.g. rhizobium.[109,110] Endophytes are a subgroup of rhizobacteria known for entering the endorhiza (the root interior) once the rhizosphere has been colonized. Moreover, they are known to show a plant growth promoting behavior more intense when compared to exclusively rhizospheric colonizing microbes.[111] The penetration process does not involve an active mechanism, but rather a passive one. Passive penetration can take place at cracks, such as those occurring at root emergence sites or created by deleterious microorganisms, as well as by root tips.[112] However, some microorganisms have developed active mechanisms, such as root nodulating rhizobia. The nodulation mechanism is mediated by root release of chemoattractants (e.g. flavonoid exudes) and microbial signals (nod factors) and as such it is specific and specialized. Root invasion can happen through fissures that occur at lateral root base and by cortical intracellular entry.[113,114] Besides, plant-rhizobia endophytic interactions are not well understood. Further, emerging but limited knowledge exists on endophytes colonizing flowers, fruit and seeds.[115] In addition, evidence of endophytic microbes found in plant stems and leaves and not in the rhizosphere highlights other potential colonization mechanisms. Bacterial endophytes are carried inside the seed (vertical transmission) and can be equally important for the evolution of the microbial community of the seedling.[116,117]

Perspective

Microbe identification remains a very important matter as we search for the best performing microbes with regards to nitrogen fixation and phosphate solubilization. These remain a matter of interest as we search for nitrogen fixing microbes for cereal crops. Cereal crops makeup a considerable percentage of the foods farmed globally. The diversity of our soils has decreased with modern agricultural practices, however PGPBs play a pivotal role in enhancing the sustainability of the agriculture system and may enable the production of better-quality food, thus promoting health and wellness.

5. Application Methods

Soil microbe delivery systems, to be effective for field-scale use, have to be designed to provide a dependable source of bacteria that survives in the soil and becomes available to crops, when needed.[43] Rhizobia application can be performed on the seed surface or directly into the soil or through plant inoculation.[43,46] Seed inoculation outnumbers soil application and depends on the requirement of the type of inoculant, the seed type and inoculant volume. The efficacy of each inoculation technique needs to be taken into account. Effects such as high temperature of a seed coater and an air seeder, high pressure, rapid drying when the inoculant is sprayed into sowing machinery and when inoculated seeds are sown under hot, dry conditions, or when seeds are treated with fungicides and herbicides potentially have large deleterious effects.[43]

Seed Inoculant: Seed Coating and Bio-priming

There is typically limited success from coating seeds with rhizobia because it is difficult to maintain living and active bacterial cells.[118] Factors such as temperature, humidity, and toxic substances all affect the survival of rhizobia in the seed-coating agent.[82] However, this is the most common and practical seed inoculation procedure. This happens because it is the easiest method to use and it requires considerably small volumes for inoculation.[82] Additionally, the standard seed coating technology has not changed in years.

Seed coating is a technique that entails the covering of a seed with a material laden with microbes to enhance seed performance and plant establishment while reducing cost, to meet the requirements in development for precision agriculture. (**Figure 4**). Historically, coating seeds has been broadly used as a cost-effective way to alleviate abiotic and biotic stresses, thus boosting crop growth, yield, and health.[119] The process is very streamlined; seeds are dusted with peat inoculant, with or without water or adhesive. With small seeds, fillers such as limestone are added, with or without adhesive, and allowed to dry.[43] The coated seeds are dried in situ or just before sowing. In situ coating standardizes the delivery and makes the technology easy to use for farmers but tends to lead to lower microbial count than coating before sowing. Seed may be a basic input deciding the fate of productivity of any crop. Commonly, seeds are studied for their germination and distributed to growers. Despite the very fact that the germination percentage registered within the seed testing laboratory is about 80-90%, these efficiency can hardly be replicated in the field because of the inadequacy or non-availability of sufficient moisture under rain fed systems.[120]

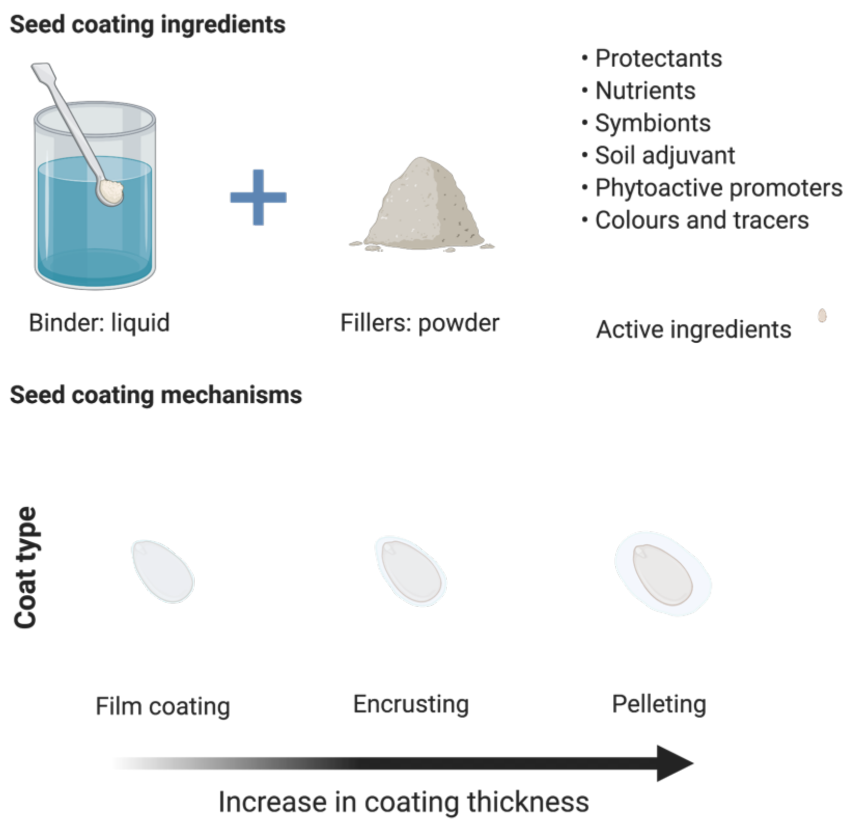

Figure 4. Seed coating ingredients, process and types.

One essential condition to seed coating is adding adhesive materials. There is no standardized material used as an adhesive.[121] Adhesives are used to ensure that a threshold of microbes are added and to secure microbes on the seed. Adhesives include gum arabic, carboxy-methyl cellulose, sucrose solutions, vegetable oils, as well as any non-toxic, commercial adhesive that can bind to bacteria and seeds.[43] With regards to seed coating applications, coating is either performed by hand, rotating drums that are cheap to operate, large dough or cement mixers, or mechanical tumbling machines.[122] Liquid inoculants are directly sprayed onto the seed before being sown once dry. The microbes can be macro or microencapsulated during the process. Microencapsulation leads to smaller particles thus larger surface area, which enhances controlled release.[123]

However, seed coating has several disadvantages. Each seed can only contain a restricted amount of inoculant, which may be a limiting factor because a threshold of bacteria may be needed for successful inoculation with most PGPBs.[43] Seed coating process may damage seeds' natural coating and alter the water or oxygen absorption properties of the seed, affecting its germination capabilities.[43] Furthermore, release and degradation properties of microbes from seed coating are important parameters to control to induce microbe colonization and combat desiccation in the soil. Some fungicides and insecticides applied to the seeds before coating may be detrimental to the inoculant, therefore seed treatments need to be carefully streamlined to avoid detrimental effects on the final product.

Bio-priming is a process of biological seed treatment that involves the soaking of seeds in any solution containing required biological compound followed by redrying the seeds, which results into start of germination process except the radicle emergence.[124] It allows the bacterial imbibition into the seed, creating ideal conditions for the bacterial inoculation and colonization in the seed and reduces the chance of desiccation and the amount of pesticide applied to the field.[124] Soaking of seeds initiates the physiological germination processes, where plumule and radicle emergence is prevented, until the seeds are provided with the right temperature and oxygen after being sown. Microbes in the seed keep on multiplying and proliferate in the spermosphere even before sowing.[124] Bio-priming leads to improved germination and seedling establishment, however it has to be done on site and can be labor intensive.[46] Given the effort required for this process, it is most appropriate for low-medium volume high value crops, such as vegetable seed.[45]

Soil Inoculant

Soil inoculation is used to release high volumes of inoculant into the soil but is time intensive, expensive and may be limited by threshold number regulations.[46,125] Soil inoculation can be achieved by adding granules in the seedbed or adding a liquid inoculant into the seedbed.[43] This process ensures that no inoculant is lost during seed planting through sowing machines. Besides, small seeds that have limited surface area can be sufficiently inoculated with enough microbes using this technique.[43] In highly mechanized farming, granular inoculants work well because the machinery for seeding commonly includes accessories for application of fertilizer and pesticide and inoculation is just one additional input during seeding.[43]

Granular forms of soil inoculant include peat, marble combined with peat, perlite, charcoal or soil aggregates. Granular inoculation enhances the chance for the inoculant to be in contact with plant roots which helps with microbe colonization and therefore effectiveness.[43] The method of soil inoculation used depends on the farmer preference. Nonetheless, it always tends to be more expensive than seed coating. The method of application is determined by the seed size, equipment availability, seed fragility, presence of insecticide and fungicide on seed surface and the cost the farmer is willing to pay.[43]

Plant inoculation

The plant microenvironment is naturally colonized by microorganisms. More than 90% are bacteria.[126] Some of them are PGPBs with the ability to enhance plant growth via providing required nutrition or increasing the availability of nutrients in an assimilable form. Plant inoculation involves the inoculation of plants through root dipping or foliar spray.[46] These techniques require large amounts of inoculant, and with regards to root dipping, plant nursery preparation is also required.[46] This highlights that the root dipping process is very time and labor intensive, which makes it unfeasible in large scale agriculture.[45] PGPBs application performed on roots or on cuttings to promote in vitro rhizogenesis is mainly performed in recalcitrant species.[127,128] They can be applied as a dipping solution or can be added to the rooting media just before transferring the shoots.[129,130]

Exogenous application using foliar spraying is conducted using the inoculum alone or in a specific formulations to ensure bacterial cells fixation on the leaves, and also to maintain live bacterial count until colonization through the stomatal apertures.[131] This method of application relies on climatic conditions; increased atmospheric temperature alters plant microbe interaction by reducing the bacterial charge and inducing intrinsic reactions in the plant by water deficits.[132] To overcome this issue, inoculant's screening based on their thermotolerance has shown great efficacy. Current findings in greenhouse studies suggest that co-application with *Bacillus cereus* and humic acid can be used in the mitigation of heat stress damage in tomato seedlings and can be commercialized as a biofertilizer.[133] But, the inoculation is also affected by humidity and rain revealing the unfeasibility of this method in large scale agriculture with certain microbe and plant types.[45] However, Fukami et al,[134] showed that foliar spray in maize and wheat improved colonization of leaves, while soil inoculations favored root and rhizosphere colonization (**Table 1**).

Table 1. Comparison table between Biofertilizers application methods

Advantages

Application method	**Comparison**	**References**
Seed inoculation		
	Advantages	
	Seed inoculation is less expensive than in-furrow inoculation, especially for small seeds	135
	Can be stored easily	136
	Low costs of storage. Easy handling and transportation	45
	Used for recalcitrant species multiplied by seeds like Orchids	137,138
Seed coating	Controlled release of microorganisms	119
	Increase of the microbial shelf life	119
	Limitations	
	Adapted to microbes compatible with dry formulations	45
	Non-sporulating bacteria experience large viable cell losses during dry formulation	75
	Affected by storage conditions	139
	Affected by the abrasion and seed contact	140
	Antagonism between the soil microbiome and the inoculated bacteria	141
Biopriming	**Advantages**	
	Useful to combat the disease problem	142,143

	Improve immediate availability of micronutrients	144
	Used for recalcitrant species	145,146
	Limitations	
	Immediate application	147
	Depend on the interaction time	147
Soil inoculation	**Advantages**	
	Increase of the effectiveness by immobilization of inoculant cells and their embodiment in polymers	148
	Limitations	
	Antagonism between the soil microbiome and the inoculated bacteria	141
Plant inoculation		
Root	**Advantages**	
	Adapted to *in vitro* plants and recalcitrant species	127,128
	Facilitate bacterial root adhesion through formation of biofilm on root surface	149
	Limitations	
	Requires large amounts of inoculant and the concentration of the bacterial suspension	150
	Depend on the exposure time of the root to the bacteria	150
Foliar	**Advantages**	
	Passive colonization through to the stomata apertures, plant wounds or insect feeding	134,151
	Can be combined to nanoparticles to increase the efficiency and the effectiveness of the inoculation	152
	Limitations	
	Unfeasibility in large scale agriculture	45
	Spraying equipment can influence the uniformity of foliar spray	153
	Depend on droplet size in terms of microbe concentration and leaf coverage	154

Seedling pretreatment	**Advantages**	
	Can be used in greenhouse vegetables	155
	Limitations	
	Requires a plasma treatment for immediate and effective bacteria activation	156

Perspective

Seed coatings provide a targeted, controlled, and low volume way to deliver beneficial microbes to the plant microbiome. An ideal strategy for future technologies consist in the development of seed coating techniques that can be streamlined in seed treatment processed and applied during the seed packaging to ensure standardization of seeds for planting. However, inoculation through seed coating formulations need to reach performances that are comparable to coating on site or soil inoculation, to have an impact in precision agriculture, despite providing an easier technology.

6. Legislation and Business Opportunity

Regulation and legislation from production to on field application of microbial fertilizers will play an important role in their use and eventual success.[157,158] Environmental policies regulate the type and quantities of microbes allowed in their environment, but also impose restrictions the type of carrier used and degradation profile permitted for each carrier. In particular, an increasing amount of attention is growing in the use of microplastics in agricultural practices, despite the low quantities involved. One of the toughest challenges for policymakers is the lack of a universally accepted definition for microbial fertilizer. The different types of microbes utilized to improve plant growth (fungi or bacteria) and the different mechanisms they used to obtain this final effect have created some inconsistencies in the definition of biofertilizers. There is then a need to develop adequate standards and legal provisions to support the production and use of biofertilizers at the global level. Globalization of microbial markets and the need for environmentally friendly and sustainable agricultural activities strengthens this need.

Recently, the European Union (EU) came up with a definition for microbial fertilizers. The new regulations will come into effect in 2022. Prior to these new regulations, the European market was segmented and now it will move into a more consolidated one. Further, this type of regulations will reduce costs and administrative burden when launching a product. Europe is the second largest biofertilizer market with 30% of the industry in 2019 and is expected to grow at 10%/year for the next several years.[159] Further, the EU defined biostimulants by what they do, not by what they are. The European Biostimulant Industry Council defines plant biostimulants as substances and/or microorganisms whose function when applied to plants or to soil is to stimulate natural processes to enhance or benefit nutrient uptake, nutrient efficiency, tolerance to abiotic stress and crop quality.[160] It is projected that this new EU regulation will improve transparency, quality and safety. Additionally, the EU set out a new procedure for authorizing biostimulants in agriculture, which will ensure conformity and accreditation in all member states. New regulations are stricter and manufacturers can only declare those benefits derived from their products that have been scientifically proven. These new requirements will provide greater transparency and confidence when defining the limits of the efficacy. However, on the innovation side, only four microorganisms are regulated, meaning any product developed from other microorganisms cannot be marketed in the EU. This highlights the growing need of aligning innovation and regulation.

In the USA, there is no federal law regulating biofertilizers. However, the individual states regulate this type of product through the United States Department of Agriculture.[158] Regulations may differ drastically, where in some states only notification is required and in some other, local efficacy trials are required. The fragmented market makes it costly and bureaucratic to operate in the US market.[161] Further, in the USA there are currently no legal definitions for the term ‘biofertilizer’, or specific legal provisions defining their characteristics.[162]

The global biofertilizers market size was USD 1.34 billion in 2018 and is projected to reach USD 3.15 billion by the end of 2026, showing a compound annual growth rate of 11.3% forecast 2019-2026.[163] With regards to application, the global fertilizer industry is segmented into seed treatment, soil treatment and other. Seed treatment has the largest market share [164] (65% in 2014) and is expected to grow by 12.1%/ year between 2019-2026. Therefore, making the seed treatment application a lucrative sector to enter. Further, nitrogen fixing biofertilizers are the leading segment in the market (82%) and is expected to remain the most important biofertilizer segment. North America and Europe account for 55% of the global market revenue. The trade in North America is expanding considerably, due to the growing number of organic farms in prominent economies, such as the U.S., Canada, and Mexico. Novozymes AS, Rizobacter Argentina S.A., Lallemand Inc., and BioWorks Inc. are the key active players in the biofertilizers business. North

America is expected to hold the highest market share in the biofertilizers market. The market is highly fragmented, with many small and large players present across different geographical regions. The global biofertilizers commerce being unregulated is the reason why there are many small companies in the market. Once proper regulations are put in place, it is likely that the market will be consolidated among a few companies.

Further, with the recent European Union ban on intentionally added microplastics (IAMPs), agriculture based companies will require to be cognizant on the type of materials manufactured for plant and soil application and thus, microbial fertilizer application tools.[165] Recently, IAMPs have become an issue of importance because of their ubiquitous presence. However, most research has been focused on the marine environment and not much on soil until of late.[166] Soils may represent a large reservoir of IAMPs, with sources such as sewage sludge applied as fertilizer and fallout from the air. Therefore, IAMPs may pose a threat to soil biodiversity. However, there is still a lack of information.[167] Recent studies, show harmful effects of IAMPs on various groups of soil fauna such as earthworms, snails, collembolans and nematodes.[168] Nevertheless, the impacts of IAMPs on soil microbial communities have led to inconsistent results.[168]

Perspective

Farming is a low margin business thus any new strategy suggested requires to be effective and cheap. Numerous effective techniques have been developed in laboratories across the world. However, collaboration between research and business is required to ensure scalability of these exciting ideas. Thus, startups working to scale up and lower costs of farming techniques will be required to bring some of the new technologies and techniques to the farmer. Also, working with government will be critical to develop supportive legislation for these initiatives.

7. Future Perspective

Climate change and rapid population growth combined with the scarcity of resources impose a rapid transformation of agriculture to a more resilient and sustainable infrastructure. Crop production is currently too carbon intensive and lower the carbon footprint of synthetic fertilizers is one of the major goals to enable a more sustainable future for our society. Microbial fertilizers have shown great potential in solving the environmental challenges we face.[169] Future formulations for microbial inoculants will focus on precise and scalable delivery tools for microbes, while also focusing on developing multi-functional microbe solutions that work for a variety of crops. However, we face a two-pronged challenge for the effective use of biofertilizers that will spur large and small-scale uptake: 1. Effective delivery methods 2a. Microbes for cereal crops 2b. Multi-functional microbe solutions. Furthermore, cost of microbial inoculants will be key to complementing with synthetic fertilizers.

Engineering the seed microenvironment with microbes in silk and trehalose seed coating has recently shown to effectively deliver plant microbial fertilizers.[83] A protein and polysaccharide mixture that encapsulated microbes was shown to be able to protect rhizobium from desiccation for over a month and finally deliver in the soil the microbes for colonization.[83] The bioinspired approach that guided the material formulation imparted the appropriate mechanical properties and preservation capabilities required for an effective microbial delivery tool. This may enable the application of the proposed seed coating technology both for small scale farmers and large-scale farmers, independently from their resources, skills and equipment. Secondly, the ability to preserve microbes at standard conditions suggests that storage costs can be lowered as most microbial fertilizers to be preserved require to be refrigerated. The framework of the technique of engineering the seed microenvironment can be used at large scale to solve the most important challenges faced in making microbial fertilizers ubiquitous in agriculture.

Cereal crop production accounts for a large proportion of agricultural production in the world providing 60% of plant calories for humans.[170,171] Therefore, corn, wheat and rice are some of the most important crops that will be essential in driving uptake of microbial fertilizers. Nitrogen based fertilizers account for more than two thirds of global revenue.[172] Recently, Pivot Bio commercialized and released nitrogen fixing microbes for corn that can supply cheaply and environmentally the necessary nitrogen in association with synthetic fertilizer, thus lowering environmental impact (**Figure 5**). From 2015, several techniques have been explored. One technique mentioned by Geddes [173], is the transfer of nitrogenase and other supporting traits to microorganisms that already closely associate with cereal crops as a logical approach to deliver nitrogen to cereal crops . Ryu et al. [174] show to engineer inducible nitrogenase activity in two cereal endophytes (*Azorhizobium caulinodans* ORS571 and *Rhizobium* sp. IRBG74) and the well-characterized plant epiphyte *Pseudomonas protegens* Pf-5, a maize seed inoculant.[174]

Such synthetic biotechnology tools have opened up possibilities for rice and wheat nitrogen fixation in the near future as highlighted by previous literature and Pivot Bio.

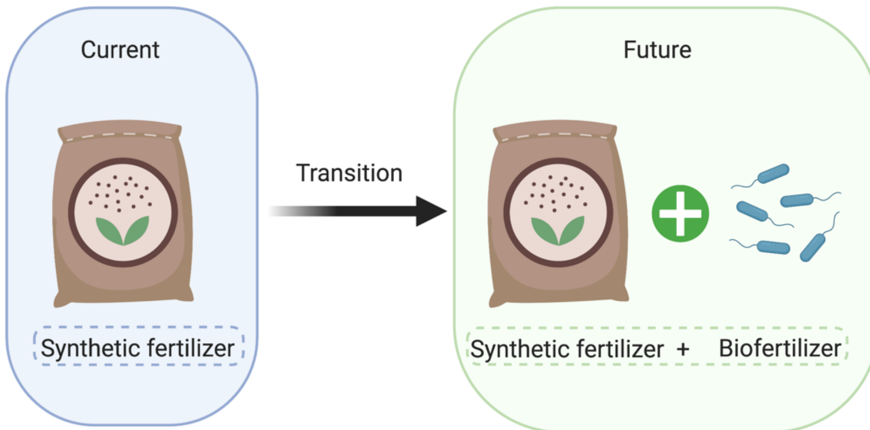

Figure 5. Transition from synthetic to microbe-based fertilizers in synergy with synthetic fertilizers to improve soil health and lower environmental impact through increasing fertilizer absorption rates thus minimizing runoff rates, solubilizing phosphates and fixing nitrogen for the plant.

Special attention is increasing for microbial inoculants that have multifunctional properties and contain more than one organism.[172] Most biofertilizers to date consist of one inoculant. However, it has been shown a consortium of microbes confer additional benefits to the plant and soil. Therefore, the drive to commercialize multifunctional property and consortium microbe fertilizers. Strains of *Rhizobium*, phosphate-solubilizing bacteria and fungi, arbuscular mycorrhizal fungi, and free-living nitrogen-fixing *Azotobacter* strains improve the nodulating ability, nitrogen content and herbage yield (up to two-fold) of subabul seedlings (*Leucaena leucocephala*), in comparison with the independent application of each component of the consortium. This use case has also led to the developing of consortium-based delivery systems, which will be an important technique in enhancing colonization and performance. Further, synthetic biology has led to the development of high-throughput tools to identify elite strains at the single nodule level with the potential to revolutionize the search for elite indigenous rhizobia. [175]

Regulation will also play a huge role in the coming years to ensure standardization of products and easier product market entrance. Since biofertilizers are not yet ubiquitous, innovators will need to work with policy makers worldwide in developing robust policies that encourage product development and protect the environment and farmers.

Acknowledgements

This work was partially supported by Office of Naval Research (Award No. N000141812258), the National Science Foundation (Award No. CMMI-1752172), the MIT Paul M. Cook Career Development Professorship, OCP S.A., and Mohammed VI Polytechnic University (UM6P)-MIT Research Program. Biorender.com was used to generate the schematics.

References

(1) Webb, P.; Benton, T. G.; Beddington, J.; Flynn, D.; Kelly, N. M.; Thomas, S. M. The Urgency of Food System Transformation Is Now Irrefutable. *Nat. Food* **2020**, *1* (10), 584–585. https://doi.org/10.1038/s43016-020-00161-0.
(2) Funabashi, M. Human Augmentation of Ecosystems: Objectives for Food Production and Science by 2045. *npj Sci. Food* **2018**, *2* (1), 16. https://doi.org/10.1038/s41538-018-0026-4.
(3) Rockström, J.; Edenhofer, O.; Gaertner, J.; DeClerck, F. Planet-Proofing the Global Food System. *Nat. Food* **2020**, *1* (1), 3–5. https://doi.org/10.1038/s43016-019-0010-4.
(4) Acevedo, M.; Pixley, K.; Zinyengere, N.; Meng, S.; Tufan, H.; Cichy, K.; Bizikova, L.; Isaacs, K.; Ghezzi-Kopel, K.; Porciello, J. A Scoping Review of Adoption of Climate-Resilient Crops by Small-Scale Producers in Low- and Middle-Income Countries. *Nat. Plants* **2020**, *6* (10), 1231–1241. https://doi.org/10.1038/s41477-020-00783-z.
(5) Wu, K.; Wang, S.; Song, W.; Zhang, J.; Wang, Y.; Liu, Q.; Yu, J.; Ye, Y.; Li, S.;

Chen, J.; Zhao, Y.; Wang, J.; Wu, X.; Wang, M.; Zhang, Y.; Liu, B.; Wu, Y.; Harberd, N. P.; Fu, X. Enhanced Sustainable Green Revolution Yield via Nitrogen-Responsive Chromatin Modulation in Rice. *Science (80-.).* **2020**, *367* (6478). https://doi.org/10.1126/science.aaz2046.

(6) Lowry, G. V.; Avellan, A.; Gilbertson, L. M. Opportunities and Challenges for Nanotechnology in the Agri-Tech Revolution. *Nat. Nanotechnol.* **2019**, *14* (6), 517–522. https://doi.org/10.1038/s41565-019-0461-7.

(7) Kulcheski, F. R.; Córrea, R.; Gomes, I. A.; De Lima, J. C.; Margis, R. NPK Macronutrients and MicroRNA Homeostasis. *Front. Plant Sci.* **2015**, *6* (June). https://doi.org/10.3389/fpls.2015.00451.

(8) Stewart, W. M.; Roberts, T. L. Food Security and the Role of Fertilizer in Supporting It. *Procedia Eng.* **2012**, *46*, 76–82. https://doi.org/10.1016/j.proeng.2012.09.448.

(9) Roberts, T. . The Role of Fertilizer in Growing the World's Food. *Better Crop.* **2009**, *93* (2), 12–15.

(10) Qing, G.; Ghazfar, R.; Jackowski, S. T.; Habibzadeh, F.; Ashtiani, M. M.; Chen, C. P.; Smith, M. R.; Hamann, T. W. Recent Advances and Challenges of Electrocatalytic N2 Reduction to Ammonia. *Chem. Rev.* **2020**, *120* (12), 5437–5516. https://doi.org/10.1021/acs.chemrev.9b00659.

(11) Smith, C.; Hill, A. K.; Torrente-Murciano, L. Current and Future Role of Haber-Bosch Ammonia in a Carbon-Free Energy Landscape †. *Energy Environ. Sci* **2020**, *13*, 331. https://doi.org/10.1039/c9ee02873k.

(12) Conley, D. J.; Paerl, H. W.; Howarth, R. W.; Boesch, D. F.; Seitzinger, S. P.; Havens, K. E.; Lancelot, C.; Likens, G. E. Ecology - Controlling Eutrophication: Nitrogen and Phosphorus. *Science (80-.).* **2009**, *323* (5917), 1014–1015. https://doi.org/10.1126/science.1167755.

(13) Sinha, E.; Michalak, A. M.; Balaji, V. Eutrophication Will Increase during the 21st Century as a Result of Precipitation Changes. *Science (80-.).* **2017**, *357* (6349), 405–408. https://doi.org/10.1126/science.aan2409.

(14) Naamala, J.; Smith, D. L. Relevance of Plant Growth Promoting Microorganisms and Their Derived Compounds, in the Face of Climate Change. *Agronomy* **2020**, *10* (8), 1179. https://doi.org/10.3390/agronomy10081179.

(15) Bhardwaj, D.; Ansari, M.; Sahoo, R.; Tuteja, N. Biofertilizers Function as Key Player in Sustainable Agriculture by Improving Soil Fertility, Plant Tolerance and Crop Productivity. *Microb. Cell Fact.* **2014**, *13* (1), 66. https://doi.org/10.1186/1475-2859-13-66.

(16) Alewell, C.; Ringeval, B.; Ballabio, C.; Robinson, D. A.; Panagos, P.; Borrelli, P. Global Phosphorus Shortage Will Be Aggravated by Soil Erosion. *Nat. Commun.* **2020**, *11* (1), 1–12. https://doi.org/10.1038/s41467-020-18326-7.

(17) Blackwell, M.; Darch, T.; Haslam, R. Phosphorus Use Efficiency and Fertilizers: Future Opportunities for Improvements. *Front. Agric. Sci. Eng.* **2019**, *6* (4), 332. https://doi.org/10.15302/J-FASE-2019274.

(18) Wan, W.; Qin, Y.; Wu, H.; Zuo, W.; He, H.; Tan, J.; Wang, Y.; He, D. Isolation and Characterization of Phosphorus Solubilizing Bacteria With Multiple Phosphorus Sources Utilizing Capability and Their Potential for Lead Immobilization in Soil. *Front. Microbiol.* **2020**, *11*, 752. https://doi.org/10.3389/fmicb.2020.00752.

(19) dos Santos, R. M.; Diaz, P. A. E.; Lobo, L. L. B.; Rigobelo, E. C. Use of Plant Growth-Promoting Rhizobacteria in Maize and Sugarcane: Characteristics and Applications. *Front. Sustain. Food Syst.* **2020**, *4*, 136. https://doi.org/10.3389/fsufs.2020.00136.

(20) Patil, H. J.; Solanki, M. K. Microbial Inoculant: Modern Era of Fertilizers and Pesticides. In *Microbial Inoculants in Sustainable Agricultural Productivity: Vol. 1: Research Perspectives*; Springer India, 2016; pp 319–343. https://doi.org/10.1007/978-81-322-2647-5_19.

(21) Geisseler, D.; Scow, K. M. Long-Term Effects of Mineral Fertilizers on Soil Microorganisms - A Review. *Soil Biol. Biochem.* **2014**, *75*, 54–63. https://doi.org/10.1016/j.soilbio.2014.03.023.

(22) Pan, Y.; Cassman, N.; De Hollander, M.; Mendes, L. W.; Korevaar, H.; Geerts, R. H. E. M.; Van Veen, J. A.; Kuramae, E. E. Impact of Long-Term N, P, K, and NPK Fertilization on the Composition and Potential Functions of the Bacterial Community in Grassland Soil. *FEMS Microbiol. Ecol.* **2014**, *90* (1), 195–205. https://doi.org/10.1111/1574-6941.12384.

(23) Li, Y.; Liu, X.; Zhang, L.; Xie, Y.; Cai, X.; Wang, S.; Lian, B. Effects of Short-Term Application of Chemical and Organic Fertilizers on Bacterial Diversity of Cornfield Soil in a Karst Area. *J. Soil Sci. Plant Nutr.* **2020**, *20* (4), 2048–2058. https://doi.org/10.1007/s42729-020-00274-2.

(24) Singh, J. S.; Pandey, V. C.; Singh, D. P. Efficient Soil Microorganisms: A New Dimension for Sustainable Agriculture and Environmental Development. *Agric. Ecosyst. Environ.* **2011**, *140* (3–4), 339–353. https://doi.org/10.1016/j.agee.2011.01.017.

(25) Backer, R.; Rokem, J. S.; Ilangumaran, G.; Lamont, J.; Praslickova, D.; Ricci, E.; Subramanian, S.; Smith, D. L. Plant Growth-Promoting Rhizobacteria: Context, Mechanisms of Action, and Roadmap to Commercialization of Biostimulants for Sustainable Agriculture. *Front. Plant Sci.* **2018**, *871*, 1473. https://doi.org/10.3389/fpls.2018.01473.

(26) Sahu, P. K.; Brahmaprakash, G. P. Formulations of Biofertilizers - Approaches and Advances. In *Microbial Inoculants in Sustainable Agricultural Productivity: Vol. 2: Functional Applications*; Springer India, 2016; pp 179–198. https://doi.org/10.1007/978-81-322-2644-4_12.

(27) Rana, K. L.; Kour, D.; Kaur, T.; Devi, R.; Yadav, A. N.; Yadav, N.; Dhaliwal, H. S.; Saxena, A. K. Endophytic Microbes: Biodiversity, Plant Growth-Promoting Mechanisms and Potential Applications for Agricultural Sustainability. *Antonie Van Leeuwenhoek* **2020**, *113* (8), 1075–1107. https://doi.org/10.1007/s10482-020-01429-y.

(28) Masciarelli, O.; Llanes, A.; Luna, V. A New PGPR Co-Inoculated with Bradyrhizobium Japonicum Enhances Soybean Nodulation. *Microbiol. Res.* **2014**, *169* (7–8), 609–615. https://doi.org/10.1016/j.micres.2013.10.001.

(29) Lugtenberg, B.; Kamilova, F. Plant-Growth-Promoting Rhizobacteria. *Annu. Rev. Microbiol.* **2009**, *63* (1), 541–556. https://doi.org/10.1146/annurev.micro.62.081307.162918.

(30) Alsharif, W.; Saad, M. M.; Hirt, H. Desert Microbes for Boosting Sustainable Agriculture in Extreme Environments. *Front. Microbiol.* **2020**, *11*.

https://doi.org/10.3389/fmicb.2020.01666.
(31) Kneip, C.; Lockhart, P.; Voß, C.; Maier, U. G. Nitrogen Fixation in Eukaryotes - New Models for Symbiosis. *BMC Evol. Biol.* **2007**, *7* (1), 55. https://doi.org/10.1186/1471-2148-7-55.
(32) Taffner, J.; Erlacher, A.; Bragina, A.; Berg, C.; Moissl-Eichinger, C.; Berg, G. What Is the Role of Archaea in Plants? New Insights from the Vegetation of Alpine Bogs. *MSphere* **2018**, *3* (3), 122–140. https://doi.org/10.1128/msphere.00122-18.
(33) Sapountzis, P.; de Verges, J.; Rousk, K.; Cilliers, M.; Vorster, B. J.; Poulsen, M. Potential for Nitrogen Fixation in the Fungus-Growing Termite Symbiosis. *Front. Microbiol.* **2016**, *7* (DEC), 1993. https://doi.org/10.3389/fmicb.2016.01993.
(34) Molina-Romero, D.; Baez, A.; Quintero-Hernández, V.; Castañeda-Lucio, M.; Fuentes-Ramírez, L. E.; Bustillos-Cristales, M. del R.; Rodríguez-Andrade, O.; Morales-García, Y. E.; Munive, A.; Muñoz-Rojas, J. Compatible Bacterial Mixture, Tolerant to Desiccation, Improves Maize Plant Growth. *PLoS One* **2017**, *12* (11). https://doi.org/10.1371/journal.pone.0187913.
(35) Sundaramoorthy, S.; Raguchander, T.; Ragupathi, N.; Samiyappan, R. Combinatorial Effect of Endophytic and Plant Growth Promoting Rhizobacteria against Wilt Disease of Capsicum Annum L. Caused by Fusarium Solani. *Biol. Control* **2012**, *60* (1), 59–67. https://doi.org/10.1016/j.biocontrol.2011.10.002.
(36) Vishwakarma, K.; Kumar, N.; Shandilya, C.; Mohapatra, S.; Bhayana, S.; Varma, A. Revisiting Plant–Microbe Interactions and Microbial Consortia Application for Enhancing Sustainable Agriculture: A Review. *Front. Microbiol.* **2020**, *11*, 560406. https://doi.org/10.3389/fmicb.2020.560406.
(37) Levy, A.; Salas Gonzalez, I.; Mittelviefhaus, M.; Clingenpeel, S.; Herrera Paredes, S.; Miao, J.; Wang, K.; Devescovi, G.; Stillman, K.; Monteiro, F.; Rangel Alvarez, B.; Lundberg, D. S.; Lu, T. Y.; Lebeis, S.; Jin, Z.; McDonald, M.; Klein, A. P.; Feltcher, M. E.; Rio, T. G.; Grant, S. R.; Doty, S. L.; Ley, R. E.; Zhao, B.; Venturi, V.; Pelletier, D. A.; Vorholt, J. A.; Tringe, S. G.; Woyke, T.; Dangl, J. L. Genomic Features of Bacterial Adaptation to Plants. *Nat. Genet.* **2018**, *50* (1), 138–150. https://doi.org/10.1038/s41588-017-0012-9.
(38) Martínez-Hidalgo, P.; Maymon, M.; Pule-Meulenberg, F.; Hirsch, A. M.; Martínez-Hidalgo, P.; Maymon, M. Engineering Root Microbiomes for Healthier Crops and Soils Using Beneficial, Environmentally Safe Bacteria. *Can. J. Microbiol.* **2019**, *65*, 20. https://doi.org/10.1139/cjm-2018-0315.
(39) Genre, A.; Lumini, E.; Per, I.; Sostenibile Delle Piante, L. P.; Franken, P.; Durazzo, A.; Todeschini, V.; Aitlahmidi, N.; Mazzucco, E.; Marsano, F.; Gosetti, F.; Robotti, E.; Bona, E.; Massa, N.; Bonneau, L.; Marengo, E.; Wipf, D.; Berta, G.; Lingua, G. Impact of Beneficial Microorganisms on Strawberry Growth, Fruit Production, Nutritional Quality, and Volatilome. *Front. Plant Sci.* **2018**, *9*, 1611. https://doi.org/10.3389/fpls.2018.01611.
(40) Zuluaga, M. Y. A.; Milani, K. M. L.; Gonçalves, L. S. A.; De Oliveira, A. L. M. Diversity and Plant Growth-Promoting Functions of Diazotrophic/N-Scavenging Bacteria Isolated from the Soils and Rhizospheres of Two Species of Solanum. *PLoS One* **2020**, *15* (1), e0227422. https://doi.org/10.1371/journal.pone.0227422.
(41) Mai-Prochnow, A.; Clauson, M.; Hong, J.; Murphy, A. B. Gram Positive and Gram

Negative Bacteria Differ in Their Sensitivity to Cold Plasma. *Sci. Rep.* **2016**, *6* (1), 1–11. https://doi.org/10.1038/srep38610.
(42) Glick, B. R. Introduction to Plant Growth-Promoting Bacteria. In *Beneficial Plant-Bacterial Interactions*; Springer International Publishing, 2020; pp 1–37. https://doi.org/10.1007/978-3-030-44368-9_1.
(43) Bashan, Y.; de-Bashan, L. E.; Prabhu, S. R.; Hernandez, J.-P. Advances in Plant Growth-Promoting Bacterial Inoculant Technology: Formulations and Practical Perspectives (1998–2013). *Plant Soil* **2014**, *378* (1–2), 1–33. https://doi.org/10.1007/s11104-013-1956-x.
(44) Meena, S. K.; Rakshit, A.; Singh, H. B.; Meena, V. S. Effect of Nitrogen Levels and Seed Bio-Priming on Root Infection, Growth and Yield Attributes of Wheat in Varied Soil Type. *Biocatal. Agric. Biotechnol.* **2017**, *12*, 172–178. https://doi.org/10.1016/j.bcab.2017.10.006.
(45) O'Callaghan, M. Microbial Inoculation of Seed for Improved Crop Performance: Issues and Opportunities. *Appl. Microbiol. Biotechnol.* **2016**, *100* (13), 5729–5746. https://doi.org/10.1007/s00253-016-7590-9.
(46) Rocha, I.; Ma, Y.; Souza-Alonso, P.; Vosátka, M.; Freitas, H.; Oliveira, R. S. Seed Coating: A Tool for Delivering Beneficial Microbes to Agricultural Crops. *Front. Plant Sci.* **2019**, *10*, 1357. https://doi.org/10.3389/fpls.2019.01357.
(47) Malusà, E.; Pinzari, F.; Canfora, L. Efficacy of Biofertilizers: Challenges to Improve Crop Production. In *Microbial Inoculants in Sustainable Agricultural Productivity: Vol. 2: Functional Applications*; Springer India, 2016; pp 17–40. https://doi.org/10.1007/978-81-322-2644-4_2.
(48) Oliveira, A. L. M.; Stoffels, M.; Schmid, M.; Reis, V. M.; Baldani, J. I.; Hartmann, A. Colonization of Sugarcane Plantlets by Mixed Inoculations with Diazotrophic Bacteria. *Eur. J. Soil Biol.* **2008**, *45*, 106–113. https://doi.org/10.1016/j.ejsobi.2008.09.004.
(49) Bonito, G.; Reynolds, H.; Robeson, M. S.; Nelson, J.; Hodkinson, B. P.; Tuskan, G.; Schadt, C. W.; Vilgalys, R. Plant Host and Soil Origin Influence Fungal and Bacterial Assemblages in the Roots of Woody Plants. *Mol. Ecol.* **2014**, *23* (13), 3356–3370. https://doi.org/10.1111/mec.12821.
(50) Saad, M. M.; Aziz Eida, A.; Hirt, H. Tailoring Plant-Associated Microbial Inoculants in Agriculture: A Roadmap for Successful Application. *J. Exp. Bot.* **2020**, *71* (13), 3878–3901. https://doi.org/10.1093/jxb/eraa111.
(51) Arora, N. K.; Verma, M.; Mishra, J. Rhizobial Bioformulations: Past, Present and Future. In *Rhizotrophs: Plant Growth Promotion to Bioremediation*; Springer Singapore, 2017; pp 69–99. https://doi.org/10.1007/978-981-10-4862-3_4.
(52) Streeter, J. G. Effect of Trehalose on Survival of Bradyrhizobium Japonicum during Desiccation. *J. Appl. Microbiol.* **2003**, *95* (3), 484–491. https://doi.org/10.1046/j.1365-2672.2003.02017.x.
(53) Roughley, R. J.; Gemell, L. G.; Thompson, J. A.; Brockwell, J. The Number of Bradyrhizobium SP. (Lupinus) Applied to Seed and Its Effect on Rhizosphere Colonization, Nodulation and Yield of Lupin. *Soil Biol. Biochem.* **1993**, *25* (10), 1453–1458. https://doi.org/10.1016/0038-0717(93)90061-F.
(54) Vílchez, J. I.; García-Fontana, C.; Román-Naranjo, D.; González-López, J.; Manzanera, M. Plant Drought Tolerance Enhancement by Trehalose Production

of Desiccation-Tolerant Microorganisms. *Front. Microbiol.* **2016**, *7* (SEP), 1577. https://doi.org/10.3389/fmicb.2016.01577.

(55) Mekala, S.; Polepongu, S. Impact of Climate Change on Soil Microbial Community. In *Plant Biotic Interactions: State of the Art*; Springer International Publishing, 2019; pp 31–41. https://doi.org/10.1007/978-3-030-26657-8_3.

(56) Klironomos, J. H.; Allen, M. F.; Rillig, M. C.; Piotrowski, J.; Makvandi-Hejad, S.; Wolfe, B. E.; Powell, J. R. Abrupt Rise in Atmospheric CO2 Overestimates Community Response in a Model Plant-Soil System. *Nature* **2005**, *433* (7026), 621–624. https://doi.org/10.1038/nature03268.

(57) Allison, S. D.; Martiny, J. B. H. Resistance, Resilience, and Redundancy in Microbial Communities. *Proc. Natl. Acad. Sci. U. S. A.* **2008**, *105* (SUPPL. 1), 11512–11519. https://doi.org/10.1073/pnas.0801925105.

(58) Bradford, M. A. Thermal Adaptation of Decomposer Communities in Warming Soils. *Front. Microbiol.* **2013**, *4* (NOV). https://doi.org/10.3389/fmicb.2013.00333.

(59) Rousk, J.; Brookes, P. C.; Bååth, E. Contrasting Soil PH Effects on Fungal and Bacterial Growth Suggest Functional Redundancy in Carbon Mineralization. *Appl. Environ. Microbiol.* **2009**, *75* (6), 1589–1596. https://doi.org/10.1128/AEM.02775-08.

(60) Rousk, J.; Bååth, E.; Brookes, P. C.; Lauber, C. L.; Lozupone, C.; Caporaso, J. G.; Knight, R.; Fierer, N. Soil Bacterial and Fungal Communities across a PH Gradient in an Arable Soil. *ISME J.* **2010**, *4* (10), 1340–1351. https://doi.org/10.1038/ismej.2010.58.

(61) Kaiser, C.; Koranda, M.; Kitzler, B.; Fuchslueger, L.; Schnecker, J.; Schweiger, P.; Rasche, F.; Zechmeister-Boltenstern, S.; Sessitsch, A.; Richter, A. Belowground Carbon Allocation by Trees Drives Seasonal Patterns of Extracellular Enzyme Activities by Altering Microbial Community Composition in a Beech Forest Soil. *New Phytol.* **2010**, *187* (3), 843–858. https://doi.org/10.1111/j.1469-8137.2010.03321.x.

(62) Fierer, N.; Leff, J. W.; Adams, B. J.; Nielsen, U. N.; Bates, S. T.; Lauber, C. L.; Owens, S.; Gilbert, J. A.; Wall, D. H.; Caporaso, J. G. Cross-Biome Metagenomic Analyses of Soil Microbial Communities and Their Functional Attributes. *Proc. Natl. Acad. Sci. U. S. A.* **2012**, *109* (52), 21390–21395. https://doi.org/10.1073/pnas.1215210110.

(63) Bashan, Y. Significance of Timing and Level of Inoculation with Rhizosphere Bacteria on Wheat Plants. *Soil Biol. Biochem.* **1986**, *18* (3), 297–301. https://doi.org/10.1016/0038-0717(86)90064-7.

(64) Hacquard, S.; Schadt, C. W. Towards a Holistic Understanding of the Beneficial Interactions across the *Populus* Microbiome. *New Phytol.* **2015**, *205* (4), 1424–1430. https://doi.org/10.1111/nph.13133.

(65) Lundberg, D. S.; Lebeis, S. L.; Paredes, S. H.; Yourstone, S.; Gehring, J.; Malfatti, S.; Tremblay, J.; Engelbrektson, A.; Kunin, V.; Rio, T. G. Del; Edgar, R. C.; Eickhorst, T.; Ley, R. E.; Hugenholtz, P.; Tringe, S. G.; Dangl, J. L. Defining the Core Arabidopsis Thaliana Root Microbiome. *Nature* **2012**, *488* (7409), 86–90. https://doi.org/10.1038/nature11237.

(66) Bonito, G.; Benucci, G. M. N.; Hameed, K.; Weighill, D.; Jones, P.; Chen, K.-H.; Jacobson, D.; Schadt, C.; Vilgalys, R. Fungal-Bacterial Networks in the Populus

Rhizobiome Are Impacted by Soil Properties and Host Genotype. *Front. Microbiol.* **2019**, *10*, 1–21. https://doi.org/10.3389/fmicb.2019.00481.
(67) Gupta, V. V. S. R.; Germida, J. J. Distribution of Microbial Biomass and Its Activity in Different Soil Aggregate Size Classes as Affected by Cultivation. *Soil Biol. Biochem.* **1988**, *20* (6), 777–786. https://doi.org/10.1016/0038-0717(88)90082-X.
(68) Juyal, A.; Eickhorst, T.; Falconer, R.; Otten, W. Effect of Soil Structure on the Growth of Bacteria in Soil Quantified Using CARD-FISH. In *EGU General Assembly*; 2014; Vol. 16, p 375.
(69) Gupta, V. Microbes and Soil Structure. In *Encyclopedia of Earth Sciences Series*; Springer Netherlands, 2011; Vol. Part 4, pp 470–472. https://doi.org/10.1007/978-90-481-3585-1_91.
(70) Foster, R. C. Microenvironments of Soil Microorganisms. *Biol. Fertil. Soils* **1988**, *6* (3), 189–203. https://doi.org/10.1007/BF00260816.
(71) Albareda, M.; Rodríguez-Navarro, D. N.; Camacho, M.; Temprano, F. J. Alternatives to Peat as a Carrier for Rhizobia Inoculants: Solid and Liquid Formulations. *Soil Biol. Biochem.* **2008**, *40* (11), 2771–2779. https://doi.org/10.1016/j.soilbio.2008.07.021.
(72) Santos, M. S.; Nogueira, M. A.; Hungria, M. Microbial Inoculants: Reviewing the Past, Discussing the Present and Previewing an Outstanding Future for the Use of Beneficial Bacteria in Agriculture. *AMB Express* **2019**, *9* (1). https://doi.org/10.1186/s13568-019-0932-0.
(73) Brockwell, J.; Bottomley, P. J. Recent Advances in Inoculant Technology and Prospects for the Future. *Soil Biol. Biochem.* **1995**, *27* (4–5), 683–697. https://doi.org/10.1016/0038-0717(95)98649-9.
(74) Jung, G.; Mugnier, J.; Diem, H. G.; Dommergues, Y. R. Polymer-Entrapped Rhizobium as an Inoculant for Legumes. *Plant Soil* **1982**, *65* (2), 219–231. https://doi.org/10.1007/BF02374652.
(75) Berninger, T.; González López, Ó.; Bejarano, A.; Preininger, C.; Sessitsch, A. Maintenance and Assessment of Cell Viability in Formulation of Non-Sporulating Bacterial Inoculants. *Microb. Biotechnol.* **2018**, *11* (2), 277–301. https://doi.org/10.1111/1751-7915.12880.
(76) Brito, B.; Palacios, J. M.; Imperial, J.; Ruiz-Argüeso, T. Engineering the Rhizobium Leguminosarum Bv. Viciae Hydrogenase System for Expression in Free-Living Microaerobic Cells and Increased Symbiotic Hydrogenase Activity. *Appl. Environ. Microbiol.* **2002**, *68* (5), 2461–2467. https://doi.org/10.1128/AEM.68.5.2461-2467.2002.
(77) Morel, M. A.; Cagide, C.; Minteguiaga, M. A.; Dardanelli, M. S.; Castro-Sowinski, S. The Pattern of Secreted Molecules during the Co-Inoculation of Alfalfa Plants with Sinorhizobium Meliloti and Delftia Sp. Strain JD2: An Interaction That Improves Plant Yield. *Mol. Plant-Microbe Interact.* **2015**, *28* (2), 134–142. https://doi.org/10.1094/MPMI-08-14-0229-R.
(78) Muñoz, N.; Soria-Díaz, M. E.; Manyani, H.; Sánchez-Matamoros, R. C.; Serrano, A. G.; Megías, M.; Lascano, R. Structure and Biological Activities of Lipochitooligosaccharide Nodulation Signals Produced by Bradyrhizobium Japonicum USDA 138 under Saline and Osmotic Stress. *Biol. Fertil. Soils* **2014**, *50* (2), 207–215. https://doi.org/10.1007/s00374-013-0843-1.

(79) Dashti, N.; Prithiviraj, B.; Zhou, X.; Hynes, R. K.; Smith, D. L. Combined Effects of Plant Growth-Promoting Rhizobacteria and Genistein on Nitrogen Fixation in Soybean at Suboptimal Root Zone Temperatures. *J. Plant Nutr.* **2000**, *23* (5), 593–604. https://doi.org/10.1080/01904160009382043.
(80) Mishra, J.; Arora, N. K. Bioformulations for Plant Growth Promotion and Combating Phytopathogens: A Sustainable Approach. In *Bioformulations: For Sustainable Agriculture*; Springer International Publishing, 2016; pp 3–33. https://doi.org/10.1007/978-81-322-2779-3_1.
(81) Morel, M. A.; Cagide, C.; Castro-Sowinski, S. The Contribution of Secondary Metabolites in the Success of Bioformulations. In *Bioformulations: For Sustainable Agriculture*; Springer International Publishing, 2016; pp 235–250. https://doi.org/10.1007/978-81-322-2779-3_13.
(82) Deaker, R.; Roughley, R. J.; Kennedy, I. R. Legume Seed Inoculation Technology—a Review. *Soil Biol. Biochem.* **2004**, *36* (8), 1275–1288. https://doi.org/10.1016/J.SOILBIO.2004.04.009.
(83) Zvinavashe, A. T.; Lim, E.; Sun, H.; Marelli, B. A Bioinspired Approach to Engineer Seed Microenvironment to Boost Germination and Mitigate Soil Salinity. *Proc. Natl. Acad. Sci. U. S. A.* **2019**, *116* (51), 25555–25561. https://doi.org/10.1073/pnas.1915902116.
(84) Sun, H.; Marelli, B. Growing Silk Fibroin in Advanced Materials for Food Security. *MRS Commun.* **2021**, 1–15. https://doi.org/10.1557/s43579-020-00003-x.
(85) Yu, Y.; Bu, F.; Zhou, H.; Wang, Y.; Cui, J.; Wang, X.; Nie, G.; Xiao, H. Biosafety Materials: An Emerging New Research Direction of Materials Science from the COVID-19 Outbreak. *Materials Chemistry Frontiers*. Royal Society of Chemistry July 1, 2020, pp 1930–1953. https://doi.org/10.1039/d0qm00255k.
(86) Kim, D.; Cao, Y.; Mariappan, D.; Bono, M. S.; Hart, A. J.; Marelli, B. A Microneedle Technology for Sampling and Sensing Bacteria in the Food Supply Chain. *Adv. Funct. Mater.* **2020**, 2005370. https://doi.org/10.1002/adfm.202005370.
(87) Ruggeri, E.; Kim, D.; Cao, Y.; Farè, S.; De Nardo, L.; Marelli, B. A Multilayered Edible Coating to Extend Produce Shelf Life. *ACS Sustain. Chem. Eng.* **2020**, *8* (38), 14312–14321. https://doi.org/10.1021/acssuschemeng.0c03365.
(88) Arora, N. K.; Khare, E.; Maheshwari, D. K. Plant Growth Promoting Rhizobacteria: Constraints in Bioformulation, Commercialization, and Future Strategies. In *Plant growth and health promoting bacteria*; 2010; pp 97–116. https://doi.org/10.1007/978-3-642-13612-2_5.
(89) Kaljeet, S.; Keyeo, F.; Amir, H. G. Influence of Carrier Materials and Storage Temperature on Survivability of Rhizobial Inoculant. *Asian J. Plant Sci.* **2011**, *10* (6), 331–337. https://doi.org/10.3923/ajps.2011.331.337.
(90) Brockwell, J.; Bottomley, P. J.; Thies, J. E. Manipulation of Rhizobia Microflora for Improving Legume Productivity and Soil Fertility: A Critical Assessment. *Plant and Soil*. Springer 1995, pp 143–180. https://doi.org/10.2307/42947553.
(91) Herrmann, L.; Atieno, M.; Brau, L.; Lesueur, D. Microbial Quality of Commercial Inoculants to Increase BNF and Nutrient Use Efficiency. In *Biological Nitrogen Fixation*; John Wiley & Sons, Inc: Hoboken, NJ, USA, 2015; Vol. 2–2, pp 1031–1040. https://doi.org/10.1002/9781119053095.ch101.

(92) Saranraj, P.; Sivasakthivelan, P. Azospirillum and Its Formulations: A Review. *Int. J. Microbiol. Res.* **2013**, *4* (3), 275–287. https://doi.org/10.5829/idosi.ijmr.2013.4.3.825.

(93) Huang, S.; Wang, L.; Liu, L.; Hou, Y.; Li, L. Nanotechnology in Agriculture, Livestock, and Aquaculture in China. A Review. *Agron. Sustain. Dev.* **2015**, *35* (2), 369–400. https://doi.org/10.1007/s13593-014-0274-x.

(94) Shukla, S. K.; Kumar, R.; Mishra, R. K.; Pandey, A.; Pathak, A.; Zaidi, M.; Srivastava, S. K.; Dikshit, A. Prediction and Validation of Gold Nanoparticles (GNPs) on Plant Growth Promoting Rhizobacteria (PGPR): A Step toward Development of Nano-Biofertilizers. *Nanotechnol. Rev.* **2015**, *4* (5), 439–448. https://doi.org/10.1515/ntrev-2015-0036.

(95) Kumar Mishra, V.; Kumar, A. Impact of Metal Nanoparticles on the Plant Growth Promoting Rhizobacteria. *Dig. J. Nanomater. Biostructures* **2009**, *4* (3), 587–592.

(96) Caraglia, M.; De Rosa, G.; Abbruzzese, A.; Leonetti, C. Nanotechnologies: New Opportunities for Old Drugs. The Case of Aminobisphosphonates. *J. Nanomedine. Biotherapeutic Discov.* **2011**, *01* (01). https://doi.org/10.4172/2155-983x.1000103e.

(97) Kumar, A.; Singh, A. K.; Choudhary, K. K. *Role of Plant Growth Promoting Microorganisms in Sustainable Agriculture and Nanotechnology*; Woodhead Publishing, 2019.

(98) Arora, N. K.; Tewari, S.; Singh, S.; Lal, N.; Maheshwari, D. K. PGPR for Protection of Plant Health under Saline Conditions. In *Bacteria in Agrobiology: Stress Management*; Springer-Verlag Berlin Heidelberg, 2012; Vol. 9783642234651, pp 239–258. https://doi.org/10.1007/978-3-642-23465-1_12.

(99) Laranjo, M.; Alexandre, A.; Oliveira, S. Legume Growth-Promoting Rhizobia: An Overview on the Mesorhizobium Genus. *Microbiol. Res.* **2014**, *169* (1), 2–17. https://doi.org/10.1016/j.micres.2013.09.012.

(100) Geetha, S. J.; Joshi, S. J. Engineering Rhizobial Bioinoculants: A Strategy to Improve Iron Nutrition. *Sci. World J.* **2013**, 315890. https://doi.org/10.1155/2013/315890.

(101) Romano, I.; Ventorino, V.; Pepe, O. Effectiveness of Plant Beneficial Microbes: Overview of the Methodological Approaches for the Assessment of Root Colonization and Persistence. *Front. Plant Sci.* **2020**, *11*. https://doi.org/10.3389/fpls.2020.00006.

(102) Walker, T. S.; Bais, H. P.; Grotewold, E.; Vivanco, J. M. Root Exudation and Rhizosphere Biology. *Plant Physiol.* **2003**, *132* (1), 44–51. https://doi.org/10.1104/pp.102.019661.

(103) Ahemad, M.; Kibret, M. Mechanisms and Applications of Plant Growth Promoting Rhizobacteria: Current Perspective. *J. King saud Univ.* **2014**, *26* (1), 1–20. https://doi.org/10.1016/j.jksus.2013.05.001.

(104) Gamalero, E.; Lingua, G.; Berta, G.; Lemanceau, P. Methods for Studying Root Colonization by Introduced Beneficial Bacteria. *Agronomie* **2003**, *23* (5–6), 407–418. https://doi.org/10.1051/agro:2003014.

(105) Compant, S.; Clément, C.; Sessitsch, A. Plant Growth-Promoting Bacteria in the Rhizo- and Endosphere of Plants: Their Role, Colonization, Mechanisms Involved and Prospects for Utilization. *Soil Biol. Biochem.* **2010**, *42* (5), 669–678.

https://doi.org/10.1016/j.soilbio.2009.11.024.
(106) Benizri, E.; Baudoin, E.; Guckert, A. Root Colonization by Inoculated Plant Growth-Promoting Rhizobacteria. *Biocontrol Sci. Technol.* **2001**, *11* (5), 557–574. https://doi.org/10.1080/09583150120076120.
(107) Whipps, J. M. Microbial Interactions and Biocontrol in the Rhizosphere. *J. Exp. Bot.* **2001**, *52* (suppl 1), 487–511. https://doi.org/10.1093/jexbot/52.suppl_1.487.
(108) Lugtenberg, B. J. J.; Dekkers, L. C. What Makes Pseudomonas Bacteria Rhizosphere Competent? *Environ. Microbiol.* **1999**, *1* (1), 9–13. https://doi.org/10.1046/j.1462-2920.1999.00005.x.
(109) Sessitsch, A.; Howieson, J. G.; Perret, X.; Antoun, H.; Martínez-Romero, E. Advances in Rhizobium Research. *CRC. Crit. Rev. Plant Sci.* **2002**, *21* (4), 323–378. https://doi.org/10.1080/0735-260291044278.
(110) Afzal, I.; Shinwari, Z. K.; Sikandar, S.; Shahzad, S. Plant Beneficial Endophytic Bacteria: Mechanisms, Diversity, Host Range and Genetic Determinants. *Microbiol. Res.* **2019**, *221*, 36–49. https://doi.org/10.1016/j.micres.2019.02.001.
(111) Dawwam, G. E.; Elbeltagy, A.; Emara, H. M.; Abbas, I. H.; Hassan, M. M. Beneficial Effect of Plant Growth Promoting Bacteria Isolated from the Roots of Potato Plant. *Ann. Agric. Sci.* **2013**, *58* (2), 195–201. https://doi.org/10.1016/j.aoas.2013.07.007.
(112) Reinhold-Hurek, B.; Hurek, T. Interactions of Gramineous Plants with Azoarcus Spp. and Other Diazotrophs: Identification, Localization, and Perspectives to Study Their Function. *CRC. Crit. Rev. Plant Sci.* **1998**, *17* (1), 29–54. https://doi.org/10.1080/07352689891304186.
(113) Garg, N.; Geetanjali. Symbiotic Nitrogen Fixation in Legume Nodules: Process and Signaling. A Review. *Agron. Sustain. Dev.* **2007**, *27* (1), 59–68. https://doi.org/10.1051/agro:2006030.
(114) Goormachtig, S.; Capoen, W.; James, E. K.; Holsters, M. Switch from Intracellular to Intercellular Invasion during Water Stress-Tolerant Legume Nodulation. *Proc. Natl. Acad. Sci. U. S. A.* **2004**, *101* (16), 6303–6308. https://doi.org/10.1073/pnas.0401540101.
(115) Compant, S.; Mitter, B.; Colli-Mull, J. G.; Gangl, H.; Sessitsch, A. Endophytes of Grapevine Flowers, Berries, and Seeds: Identification of Cultivable Bacteria, Comparison with Other Plant Parts, and Visualization of Niches of Colonization. *Microb. Ecol.* **2011**, *62* (1), 188–197. https://doi.org/10.1007/s00248-011-9883-y.
(116) Truyens, S.; Weyens, N.; Cuypers, A.; Vangronsveld, J. Bacterial Seed Endophytes: Genera, Vertical Transmission and Interaction with Plants. *Environ. Microbiol. Rep.* **2015**, *7* (1), 40–50. https://doi.org/10.1111/1758-2229.12181.
(117) Zhou, X.; Wang, J.-T.; Zhang, Z.-F.; Li, W.; Chen, W.; Cai, L. Microbiota in the Rhizosphere and Seed of Rice From China, With Reference to Their Transmission and Biogeography. *Front. Microbiol.* **2020**, *11*, 995. https://doi.org/10.3389/fmicb.2020.00995.
(118) Zhou, J.; Deng, B.; Zhang, Y.; Cobb, A. B.; Zhang, Z. Molybdate in Rhizobial Seed-Coat Formulations Improves the Production and Nodulation of Alfalfa. *PLoS One* **2017**, *12* (1), e0170179. https://doi.org/10.1371/journal.pone.0170179.
(119) Ma, Y. Seed Coating with Beneficial Microorganisms for Precision Agriculture. *Biotechnol. Adv.* **2019**, 107423. https://doi.org/10.1016/j.biotechadv.2019.107423.

(120) Tarafdar, J.; Subramaniam, K. S. Prospects of Nanotechnology in Indian Farming. *Indian J. Agric. Sci.* **2011**, *81*, 887–893.

(121) Albareda, M.; Rodríguez-Navarro, D. N.; Camacho, M.; Temprano, F. J. Alternatives to Peat as a Carrier for Rhizobia Inoculants: Solid and Liquid Formulations. *Soil Biol. Biochem.* **2008**, *40* (11), 2771–2779. https://doi.org/10.1016/j.soilbio.2008.07.021.

(122) Schulz, T. J.; Thelen, K. D. Soybean Seed Inoculant and Fungicidal Seed Treatment Effects on Soybean. *Crop Sci.* **2008**, *48* (5), 1975–1983. https://doi.org/10.2135/cropsci2008.02.0108.

(123) John, R. P.; Tyagi, R. D.; Brar, S. K.; Surampalli, R. Y.; Prévost, D. Bio-Encapsulation of Microbial Cells for Targeted Agricultural Delivery. *Crit. Rev. Biotechnol.* **2011**, *31* (3), 211–226. https://doi.org/10.3109/07388551.2010.513327.

(124) Mahmood, A.; Can Turgay, guz; Farooq, M.; Hayat, R. Seed Biopriming with Plant Growth Promoting Rhizobacteria: A Review. *FEMS Microbiol. Ecol.* **2016**, *92*, 112. https://doi.org/10.1093/femsec/fiw112.

(125) Singh, R.; Arora, N. K. Bacterial Formulations and Delivery Systems against Pests in Sustainable Agro-Food Production. *Food Sci.* **2016**. https://doi.org/10.1016/b978-0-08-100596-5.03068-7.

(126) Kim, Y. C.; Glick, B. R.; Bashan, Y.; Ryu, C. M. Enhancement of Plant Drought Tolerance by Microbes. In *Plant Responses to Drought Stress: From Morphological to Molecular Features*; Springer-Verlag Berlin Heidelberg, 2012; Vol. 9783642326530, pp 383–413. https://doi.org/10.1007/978-3-642-32653-0_15.

(127) Orlikowska, T.; Nowak, K.; Reed, B. Bacteria in the Plant Tissue Culture Environment. *Plant Cell. Tissue Organ Cult.* **2017**, *128* (3), 487–508. https://doi.org/10.1007/s11240-016-1144-9.

(128) Perez-Rosales, E.; Alcaraz-Meléndez, L.; Puente, M. E.; Vázquez-Juárez, R.; Zenteno-Savín, T.; Morales-Bojórquez, E. Endophytic Bacteria Isolated from Wild Jojoba [Simmondsia Chinensis L. (Schneider)] Roots Improve in Vitro Propagation. *Plant Cell. Tissue Organ Cult.* **2018**, *135* (3), 515–522. https://doi.org/10.1007/s11240-018-1483-9.

(129) Larraburu, E. E.; Apóstolo, N. M.; Llorente, B. E. Anatomy and Morphology of Photinia (Photinia × Fraseri Dress) in Vitro Plants Inoculated with Rhizobacteria. *Trees - Struct. Funct.* **2010**, *24* (4), 635–642. https://doi.org/10.1007/s00468-010-0433-x.

(130) Larraburu, E. E.; Yarte, M. E.; Llorente, B. E. Azospirillum Brasilense Inoculation, Auxin Induction and Culture Medium Composition Modify the Profile of Antioxidant Enzymes during in Vitro Rhizogenesis of Pink Lapacho. *Plant Cell. Tissue Organ Cult.* **2016**, *127* (2), 381–392. https://doi.org/10.1007/s11240-016-1060-z.

(131) Tamreihao, K.; Ningthoujam, D. S.; Nimaichand, S.; Singh, E. S.; Reena, P.; Singh, S. H.; Nongthomba, U. Biocontrol and Plant Growth Promoting Activities of a Streptomyces Corchorusii Strain UCR3-16 and Preparation of Powder Formulation for Application as Biofertilizer Agents for Rice Plant. *Microbiol. Res.* **2016**, *192*, 260–270. https://doi.org/10.1016/j.micres.2016.08.005.

(132) Hatfield, J. L.; Prueger, J. H. Temperature Extremes: Effect on Plant Growth and

Development. *Weather Clim. Extrem.* **2015**, *10*, 4–10. https://doi.org/10.1016/j.wace.2015.08.001.

(133) Khan, M. A.; Asaf, S.; Khan, A. L.; Jan, R.; Kang, S.-M.; Kim, K.-M.; Lee, I.-J. Extending Thermotolerance to Tomato Seedlings by Inoculation with SA1 Isolate of Bacillus Cereus and Comparison with Exogenous Humic Acid Application. *PLoS One* **2020**, *15* (4), e0232228. https://doi.org/10.1371/journal.pone.0232228.

(134) Fukami, J.; Nogueira, M. A.; Araujo, R. S.; Hungria, M. Accessing Inoculation Methods of Maize and Wheat with Azospirillum Brasilense. *AMB Express* **2016**, *6* (1), 1–13. https://doi.org/10.1186/s13568-015-0171-y.

(135) Kaminsky, L. M.; Trexler, R. V.; Malik, R. J.; Hockett, K. L.; Bell, T. H. The Inherent Conflicts in Developing Soil Microbial Inoculants. *Trends Biotechnol.* **2019**, *37* (2), 140–151. https://doi.org/10.1016/j.tibtech.2018.11.011.

(136) Swaminathan, J.; van Koten, C.; Henderson, H. V.; Jackson, T. A.; Wilson, M. J. Formulations for Delivering *Trichoderma Atroviridae* Spores as Seed Coatings, Effects of Temperature and Relative Humidity on Storage Stability. *J. Appl. Microbiol.* **2016**, *120* (2), 425–431. https://doi.org/10.1111/jam.13006.

(137) Huehne, P. S.; Bhinija, K. Application of Cryoprotectants to Improve Low Temperature Storage Survival of Orchid Seeds. *Sci. Hortic. (Amsterdam).* **2012**, *135*, 186–193. https://doi.org/10.1016/j.scienta.2011.11.026.

(138) A Uthairatanakij, J. T. da S. K. O. W. Chitosan for Improving Orchid Production and Quality. *Orchid Sci Biotechnol* **2007**, *1*, 1–5.

(139) Ali, M. A.; Ilyas, F.; Arshad, M.; Hussain, S.; Iqbal, M.; Ahmad, S.; Saboor, A.; Mustafa, G.; Ahmed, N. Microbial Inoculation of Seeds for Better Plant Growth and Productivity. In *Priming and Pretreatment of Seeds and Seedlings*; Springer Singapore, 2019; pp 523–550. https://doi.org/10.1007/978-981-13-8625-1_26.

(140) Afzal, I.; Kamran, M.; Ahmed Basra, S. M.; Ullah Khan, S. H.; Mahmood, A.; Farooq, M.; Tan, D. K. Y. Harvesting and Post-Harvest Management Approaches for Preserving Cottonseed Quality. *Ind. Crops Prod.* **2020**, *155*, 112842. https://doi.org/10.1016/j.indcrop.2020.112842.

(141) Timmusk, S.; Behers, L.; Muthoni, J.; Muraya, A.; Aronsson, A.-C. Perspectives and Challenges of Microbial Application for Crop Improvement. *Front. Plant Sci.* **2017**, *8*, 49. https://doi.org/10.3389/fpls.2017.00049.

(142) Mustafa, G.; Masood, S.; Ahmed, N.; Saboor, A.; Ahmad, S.; Hussain, S.; Bilal, M.; Ali, M. A. Seed Priming for Disease Resistance in Plants. In *Priming and Pretreatment of Seeds and Seedlings*; Springer Singapore, 2019; pp 333–362. https://doi.org/10.1007/978-981-13-8625-1_16.

(143) Shakeel, M. T.; Parveen, R.; Haider, I.; Arshad, M.; Ahmad, S.; Ahmad, N.; Hussain, S.; Riaz, M.; Ali, M. A. Seed Pretreatment as a Means to Achieve Pathogen Control. In *Priming and Pretreatment of Seeds and Seedlings*; Springer Singapore, 2019; pp 363–371. https://doi.org/10.1007/978-981-13-8625-1_17.

(144) Rehman, A.; Farooq, M.; Naveed, M.; Nawaz, A.; Shahzad, B. Seed Priming of Zn with Endophytic Bacteria Improves the Productivity and Grain Biofortification of Bread Wheat. *Eur. J. Agron.* **2018**, *94*, 98–107. https://doi.org/10.1016/j.eja.2018.01.017.

(145) Alibrandi, P.; Lo Monaco, N.; Calevo, J.; Voyron, S.; Puglia, A. M.; Cardinale, M.; Perotto, S. Plant Growth Promoting Potential of Bacterial Endophytes from Three

Terrestrial Mediterranean Orchid Species. *Plant Biosyst.* **2020**. https://doi.org/10.1080/11263504.2020.1829731.
(146) Herrera, H.; Sanhueza, T.; Novotná, A.; Charles, T. C.; Arriagada, C. Isolation and Identification of Endophytic Bacteria from Mycorrhizal Tissues of Terrestrial Orchids from Southern Chile. *Diversity* **2020**, *12* (2), 55. https://doi.org/10.3390/d12020055.
(147) Waqas, M.; Korres, N. E.; Khan, M. D.; Nizami, A.-S.; Deeba, F.; Ali, I.; Hussain, H. Advances in the Concept and Methods of Seed Priming. In *Priming and Pretreatment of Seeds and Seedlings*; Springer Singapore, 2019; pp 11–41. https://doi.org/10.1007/978-981-13-8625-1_2.
(148) Oliveira, A. L. M.; Santos, O. J. A. P.; Marcelino, P. R. F.; Milani, K. M. L.; Zuluaga, M. Y. A.; Zucareli, C.; Gonçalves, L. S. A. Maize Inoculation with Azospirillum Brasilense Ab-V5 Cells Enriched with Exopolysaccharides and Polyhydroxybutyrate Results in High Productivity under Low N Fertilizer Input. *Front. Microbiol.* **2017**, *8* (SEP). https://doi.org/10.3389/fmicb.2017.01873.
(149) Pagnani, G.; Galieni, A.; Stagnari, F.; Pellegrini, M.; Del Gallo, M.; Pisante, M. Open Field Inoculation with PGPR as a Strategy to Manage Fertilization of Ancient Triticum Genotypes. *Biol. Fertil. Soils* **2020**, *56* (1), 111–124. https://doi.org/10.1007/s00374-019-01407-1.
(150) Kurdish, I. K.; Bega, Z. T.; Gordienko, A. S.; Dyrenko, D. I. The Effect of Azotobacter Vinelandii on Plant Seed Germination and Adhesion of These Bacteria to Cucumber Roots. *Appl. Biochem. Microbiol.* **2008**, *44* (4), 400–404. https://doi.org/10.1134/S000368380804011X.
(151) Preininger, C.; Sauer, U.; Bejarano, A.; Berninger, T. Concepts and Applications of Foliar Spray for Microbial Inoculants. *Appl. Microbiol. Biotechnol.* **2018**, *102* (17), 7265–7282. https://doi.org/10.1007/s00253-018-9173-4.
(152) Ghormade, V.; Deshpande, M. V.; Paknikar, K. M. Perspectives for Nano-Biotechnology Enabled Protection and Nutrition of Plants. *Biotechnol. Adv.* **2011**, *29* (6), 792–803. https://doi.org/10.1016/j.biotechadv.2011.06.007.
(153) Bueno, M. R.; Cunha, J. P. A. R. d.; de Santana, D. G. Assessment of Spray Drift from Pesticide Applications in Soybean Crops. *Biosyst. Eng.* **2017**, *154*, 35–45. https://doi.org/10.1016/j.biosystemseng.2016.10.017.
(154) Bejarano, A.; Sauer, U.; Preininger, C. Design and Development of a Workflow for Microbial Spray Formulations Including Decision Criteria. *Appl. Microbiol. Biotechnol.* **2017**, *101* (19), 7335–7346. https://doi.org/10.1007/s00253-017-8447-6.
(155) Dursun, A.; Ekinci, M.; Dönmez, M. F. Effects of Foliar Application of Plant Growth Promoting Bacterium on Chemical Contents, Yield and Growth of Tomato (Lycopersicon Esculentum L.) and Cucumber (Cucumis Sativus L.). *Pak. J. Bot* **2010**, *42* (5), 3349–3356.
(156) Ji, S. H.; Yoo, S.; Choi, E. H.; Oh, J.; Kim, S. B. Activation of Endophytic Bacteria Useful for Plants by Atmospheric Plasma Treatment. *J. Phys. D. Appl. Phys.* **2020**, *53* (49), 494002. https://doi.org/10.1088/1361-6463/aba92e.
(157) Barros-Rodríguez, A.; Rangseekaew, P.; Lasudee, K.; Pathom-aree, W.; Manzanera, M. Regulatory Risks Associated with Bacteria as Biostimulants and Biofertilizers in the Frame of the European Regulation (EU) 2019/1009. *Sci. Total

Environ. **2020**, *740*, 140239. https://doi.org/10.1016/j.scitotenv.2020.140239.
(158) Du Jardin, P. Plant Biostimulants: Definition, Concept, Main Categories and Regulation. *Sci. Hortic. (Amsterdam).* **2015**, *196*, 3–14. https://doi.org/10.1016/j.scienta.2015.09.021.
(159) Europe Biofertilizers Market | Growth, Trends, and Forecast (2020-2025) https://mordorintelligence.com/industry-reports/europe-biofertilizers-market (accessed Nov 18, 2019).
(160) Biostimulants in European Fertilising Products Regulation.
(161) Lugtenberg, B. *Principles of Plant-Microbe Interactions: Microbes for Sustainable Agriculture*; Springer International Publishing, 2015. https://doi.org/10.1007/978-3-319-08575-3.
(162) Malusá, E.; Vassilev, N. A Contribution to Set a Legal Framework for Biofertilisers. *Appl. Microbiol. Biotechnol.* **2014**, *98* (15), 6599–6607. https://doi.org/10.1007/s00253-014-5828-y.
(163) Biofertilizers Market Size, Share | Global Analysis Report 2026 https://www.fortunebusinessinsights.com/industry-reports/biofertilizers-market-100413 (accessed Apr 3, 2019).
(164) Biofertilizers Market Size, Share & Growth Report, 2020-2027 https://www.grandviewresearch.com/industry-analysis/biofertilizers-industry (accessed Nov 18, 2019).
(165) Clausen, L. P. W.; Hansen, O. F. H.; Oturai, N. B.; Syberg, K.; Hansen, S. F. Stakeholder Analysis with Regard to a Recent European Restriction Proposal on Microplastics. *PLoS One* **2020**, *15* (6), e0235062. https://doi.org/10.1371/journal.pone.0235062.
(166) Wang, J.; Liu, X.; Li, Y.; Powell, T.; Wang, X.; Wang, G.; Zhang, P. Microplastics as Contaminants in the Soil Environment: A Mini-Review. *Sci. Total Environ.* **2019**, *691*, 848–857. https://doi.org/10.1016/j.scitotenv.2019.07.209.
(167) Boots, B.; Russell, C. W.; Green, D. S. Effects of Microplastics in Soil Ecosystems: Above and Below Ground. **2019**. https://doi.org/10.1021/acs.est.9b03304.
(168) Lin, D.; Yang, G.; Dou, P.; Qian, S.; Zhao, L.; Yang, Y.; Fanin, N. Microplastics Negatively Affect Soil Fauna but Stimulate Microbial Activity: Insights from a Field-Based Microplastic Addition Experiment. *Proc. R. Soc. B Biol. Sci.* **2020**, *287* (1934), 20201268. https://doi.org/10.1098/rspb.2020.1268.
(169) Gou, J. Y.; Suo, S. Z.; Shao, K. Z.; Zhao, Q.; Yao, D.; Li, H. P.; Zhang, J. L.; Rensing, C. Biofertilizers with Beneficial Rhizobacteria Improved Plant Growth and Yield in Chili (Capsicum Annuum L.). *World J. Microbiol. Biotechnol.* **2020**, *36* (6), 86. https://doi.org/10.1007/s11274-020-02863-w.
(170) Price, R. K.; Welch, R. W. Cereal Grains. In *Encyclopedia of Human Nutrition*; Elsevier Inc., 2012; Vol. 1–4, pp 307–316. https://doi.org/10.1016/B978-0-12-375083-9.00047-7.
(171) Awika, J. M. Major Cereal Grains Production and Use around the World. In *ACS Symposium Series*; American Chemical Society, 2011; Vol. 1089, pp 1–13. https://doi.org/10.1021/bk-2011-1089.ch001.
(172) 3. Future perspective of biofertilizers - Page 4 https://www.bio-fit.eu/q9/lo10-bio-fertilizers-technology-—-awareness,-marketing-and-future?start=3 (accessed Dec

4, 2019).

(173) Geddes, B. A.; Ryu, M. H.; Mus, F.; Garcia Costas, A.; Peters, J. W.; Voigt, C. A.; Poole, P. Use of Plant Colonizing Bacteria as Chassis for Transfer of N2-Fixation to Cereals. *Curr. Opin. Biotechnol.* **2015**, *32*, 216–222. https://doi.org/10.1016/j.copbio.2015.01.004.

(174) Ryu, M. H.; Zhang, J.; Toth, T.; Khokhani, D.; Geddes, B. A.; Mus, F.; Garcia-Costas, A.; Peters, J. W.; Poole, P. S.; Ané, J. M.; Voigt, C. A. Control of Nitrogen Fixation in Bacteria That Associate with Cereals. *Nat. Microbiol.* **2020**, *5* (2), 314–330. https://doi.org/10.1038/s41564-019-0631-2.

(175) Mendoza-Suárez, M. A.; Geddes, B. A.; Sánchez-Cañizares, C.; Ramírez-González, R. H.; Kirchhelle, C.; Jorrin, B.; Poole, P. S. Optimizing Rhizobium-Legume Symbioses by Simultaneous Measurement of Rhizobial Competitiveness and N2 Fixation in Nodules. *Proc. Natl. Acad. Sci. U. S. A.* **2020**, *117* (18), 9822–9831. https://doi.org/10.1073/pnas.1921225117.

Table of Contents Graphic