Microbials In Agriculture: A Current Review on the Perspectives and Challenges for Large Scale Implementation

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Abstract— *Growing populations, food demand and climate change necessitates the improvement of agriculture in sustainable ways. Microbials provide for an efficient green solution, and a potential replacement for the overuse of chemical pesticides. They have long been researched for their various beneficial effects in crop protection such as improving plant growth, stress tolerance and abetting plant pathogens. Despite their several advantages, the large-scale implementation of microbials is still at its primitive stage. This review attempts to identify the challenges that are barring the improvement of the microbials industry. Both the research and industry sections are explored, to recognize key issues, chokeholds and identify areas of improvement. This review provides a current and updated perspective into the use of microbials in agriculture.*

Keywords— Sustainable Agriculture, Microbials, Biofertilizers, Biopesticides, Market.

I. INTRODUCTION

The Food and Agriculture Organization (FAO) estimates the world's population to reach about 8.5 billion people in 2025 (1). Due to this rapid growth rate in population (approximately 1.05% per year), malnutrition has emerged in roughly 2.4 billion individuals across the world (2). Global food production will need to increase by at least 60% if food security is to be achieved by the year 2050 (3). In addition, an increase in agricultural productivity is also essential in realizing numerous sustainable development goals (SDG) including zero hunger (SDG 2), no poverty (SDG 1), and good health and well-being (SDGs 1 and 2), which can all ultimately benefit life on land (4). It is thus important to upgrade existing cropping systems, despite present limitations of limited resources and reduction in arable land.

Agricultural production, however, faces a number of unforeseen environmental issues such as drought, heat waves and flooding. More significantly, plant pests and pathogens cause a range of plant diseases significantly reducing agricultural output (5). For example, farmers face major food losses annually, ranging from 21.5% in wheat, 30.3% in rice, 22.6% in maize, 17.2% in potato, and 21.4% in soybean, due to pests (5). Downstream effects to human health are inevitable due to decreased yields, loss of species variety and increase in pest mitigation costs (5). Thus, along with an increase in production, a major reduction in food loss due to pests and pathogens is also required.

Currently, agrochemicals are used to increase yields and scale up crop productivity with the goal of intensive farming (4). This is done in attempt to increase agricultural production on current agricultural land, rather than an expansion in arable surface. Farmers rely on traditional agricultural practices which use inorganic fertilizers, pesticides, and other chemical inputs, as they substantially increase yield without the need of more land. Among all, phosphorus and nitrogen fertilizers are commonly used, in combination with herbicides and pesticides to help maintain crop productivity and yields, in addition to managing invasive plants, diseases, and insects (4).

The continuous and excessive use of agrochemicals, however, has led to increased soil salinity and toxicity, hardening of soil, decreased nutrient carrying capacity and water logging (6,7). Additional issues in the form of pesticide resistance prompting the use of higher doses which lead to aggressiveness of disease and pathogen mutations, have pronounced negative environmental impacts and serious implications for food security (7). Not only do they have direct effects on the environment, but they can also be damaging to human health indirectly or directly. Chemical pesticides find their way into drinking water systems and food products, exposing humans to high levels of toxicity. According to a study published in 2014, many chemical pesticides used throughout the world are far more harmful to human health and the environment than previously assumed (7). A well-known example is DDT which was the first synthetic insecticide to be produced. DDT is highly effective against insects and plant pathogens but was recently suspected to be a probable human carcinogen (7). Numerous other studies have linked chemical pesticides to cancer, Alzheimer’s disease, ADHD and birth defects (7). In addition to this, the rising cost of pesticides particularly in less developed countries, and customer demand for pesticide-free food has prompted a search for alternatives. Many fastidious diseases also do not have chemical solutions as they are ineffective or do not exist.

Thus, for all the above reasons and disadvantages of existing farming systems, it is necessary to shift to using of sustainable practices for increased crop output. Sustainable practices have the potential to offer long-term solutions to secure global food security (7). One of the most promising sustainable practices that is being implemented today is the use of microbials for crop growth promotion/ protection. Microbials are a class of biologicals that use living microorganisms for crop protection (6,7). It is a form of biological control that is preventative and/or curative in direct pest control. In addition to crop protection, they work together with the plant microbiome to help improve overall plant growth, nutrient efficiency, and stress tolerance (4,6). Microbial products contain organisms from most microorganism genera (viruses, bacteria, fungal pathogens, yeast and protozoa) (11). Bacteria are the most commonly used microbials due to their lower costs and ease of usage as compared to fungal biological control agents (11).

Microbial products have the potential to boost crop yields and supplement or replace agricultural chemicals and fertilizers (4,6,11). As opposed to chemical pesticides, microbials are made from naturally occurring materials (11). It presents as a very appealing alternative because it would drastically minimize the consumption of agrochemicals. Many companies have begun to use single or multiple microorganisms as biocontrol or biofertilizer products, as well as develop carrier-based inoculants of beneficial strains (12). Large-scale field trials have shown an improvement in crop yield of 10–20% on commercially important crop plants (11). Currently, microbials make up the largest part of the biologicals market and are expected to grow up to 60% of the biological control market by 2025 (12).

Growing consumer interest in organic agriculture, the reduction of synthetic products, and the economic potential of rising countries like India are all major growth factors in the development of the microbials market (14). However, despite the benefits and potential of agricultural microbial products, according to a recent study on microbials, "the scientific literature abounds with numerous potentially highly helpful strains that did not arrive on the commercial market" (15). It was found that, approximately 72% of biocontrol company endeavors failed over a 30-year period ending in 2002 (16).

This can be attributed to many challenges faced in the development of microbial products. Usually, the development of a commercial microbial product is a lengthy process that necessitates a high level of expertise and close collaboration among experts from numerous domains (13). The research domain includes strain isolation, efficiency testing in vitro and in vivo, and trials in natural settings. Following this, the product must be produced on a commercial scale, conserved for storage, and prepared to assure biocompatibility in order to be delivered commercially. Only then, these procedures could be patented for large scale implementation. Even the registration process appears to be an issue as, large number of patents are issued, but only a few products have been registered (6,13). Failures are also caused by underestimating the expenses of creating and marketing microbial goods. The disparity between effective microbial strains and profitable agricultural products shows that unexpected challenges must be overcome.

Microbes will undoubtedly play a role in agricultural revolution in the coming decades, helping to fulfill the needs of a growing population. In order to realize the actual potential of microbes in agriculture, more research is required to improve industry standard of microbials and commercialization. Research and industry implementation always go hand in hand. In this review, we will discuss the major challenges faced by microbial production. To highlight this, a main research question with two sub questions is formulated to delve deeper into how these challenges are currently being overcome, in addition to the current microbials market. What are the challenges faced by translation of microbial products from lab to field? What is the importance of using microbials in enhancing plant growth promotion and crop protection? What are the existing solutions to the current challenges and future perspectives? While some reviews focus either on the research or industry side, this review aims to combine both as they would go hand in hand for ultimate improvement.

II. RESEARCH METHODOLOGY

The review sought to identify, discuss and synthesize recent research into the use of microbials in crop protection. First, literature research in the field using key words: microbials, crop protection, PGPR, challenges, benefits were performed to identify a problem field to discuss. Since the field of using microbials itself is very recent, for the first section indicating their significance, literature from 1900s to recent is included. For the second section, recent literature from the years 2015-2022 was identified and used.

The inclusion criteria for the articles and research studies were, they have to be (i) full-text paper (either pre-print or published in peer-reviewed journal) (ii) English language (iii) focus on microbials in agriculture (either primary or non-primary research papers).

For the search itself, a simple key-word strategy was used for the particular sections that are needed for the main body. For example, for the section discussing the Global PGPB/PGPR market, the text strings "global microbials market AND challenges OR forecast periods" was entered into the Google search engine to obtain essential results for this review article. The search results were then screened in terms of most relevant to answer the research questions posed in this review article.

III. HISTORY OF MICROBIALS

The idea of utilizing microbes was first proposed almost 150 years ago (17). In a study conducted in 1879, Hagen proposed the dispersal of a disease-causing bacterium on crops to minimize pests (17). In addition to biocontrol, microbials as biofertility inoculants were also used. The commercial application of microbials in biofertilization dates back to Nobbe and Hiltner's (1896) development of a bacterial product named "Nitrogin" to improve agricultural yield of legume crops (18). ''Alnit"" was later discovered by Timonin (1948), which contained *Azotobacter* bacterial compounds to boost agricultural yield of non-legume crops (19).

Hagen as the earliest agromicrobe adopter in 1879 had already stressed particularly on the ease of manufacture, low cost of microbials, and the fact that it is not hazardous to human health (17,20). These factors are still significant today when choosing crop protection products. Following this framework, many other products came to the market. The use of fungal species was first introduced by Metchnikoff in 1880, when he succeeded in the artificial control of *Metharhizium anisopliae*, a pathogenic fungus to reptiles. This was later used in field trials for the control of several insect pathogens (17). Shortly followed the introduction of two bacteria that were used as insect pathogens in the early twentieth century (21). They were later produced and commercialized as the first biopesticide 'Sporein' in 1938, France (20). The first large scale commercially available biopesticides were available in Europe and USA by the 1960s, when agrochemical companies started making significant investments. Early sales in the biocontrol sector were dominated by a single product type containing *Bacillus thuringiensis* (Bt) (6). This product was specifically focused against lepidopterans (e.g., cabbage worms and gypsy moth) (22). Bt product is known as the most used microbial pesticide in world. The estimated total sales for microbial based biocontrol products were close to $400 million USD in 2012, with almost 50% of sales accounted for by Bt-related products (16, 22). Biological control became the fastest growing segment in the plant protection market as companies recognized the advantages of using microbials over chemicals. With a total sales reaching three billion in 2016 and a compound annual growth level of 16%; sales are forecasted to grow to 13.9 billion dollars by 2025, whilst also achieving a pesticide global market share of 29.9% (12).

Over the last two decades, the geographical distribution of biocontrol sales has shifted considerably to cover a larger worldwide market and a higher variety of agricultural crops (16,22). These patterns could be attributed to the expansion of the geographic scope, market sectors, key arable crops, and microbial strain diversity. In addition, growing consumer interest for microbial products in emerging markets such as China and India are major drivers of adoption (6). However, even with continued market growth, the total use remains a fraction of the total worldwide pesticide use. (6,7,13) In the later part of the review, we report on some of the key challenges that are encountered in bringing microbial products to the market.

IV. OVERVIEW AND SIGNIFICANCE OF MICROBIALS IN CURRENT AGRICULTURE

Bacteria, fungi and viruses are the major groups of microorganisms that have been found associated to plants (11). They each benefit the plant in their own way, but also sustain interrelationships between themselves. Traditionally, agricultural application of beneficial microorganisms involved a few types of well characterized microbes such as mycorrhizal fungi or rhizobia bacteria (24). The main group of microorganisms that were found to benefit plants and maintain a long lasting symbiosis were bacteria (11). They are present widely in the plant ecosystem and help the plant in both biocontrol and biofertilization, as opposed to some groups of fungi and virus (that aid mostly in uptake of nutrients). Furthermore, bacteria also participate in complex communities by forming communities through the production of signalling molecules that attract other beneficial microbial communities (4,6,11). They also continuously form biofilms or other networks, that serve as communication or transport networks, benefitting both the plant-host and the microbe (11). The diversity in plant associated microbiomes is touched upon in the next section.

Majority of research studies are focused solely on the ability of the applied microorganisms in the facilitation of specific plant growth promoting traits such as phosphate solubilization, nitrogen fixation and ACC deaminase production. siderophore production, biofilm formation, plant hormone production, biotic, and abiotic stress tolerance or resistance, amongst others (4,6,11). These microorganisms however are usually studied in small, one-on-one investigations in sterile soils and greenhouses (24). The beneficial effects are often not be observed in field situations as they fail to translate in more complex environments (24). This could be attributed to the fact that soil in field plots have more complex microbial communities that are adapted to local eco-environments (24).

V. DIVERSITY OF PLANT MICROBIOME

Diverse microorganisms can colonize different surfaces of the plant; for example- root surfaces (rhizosphere), other aerial parts- phyllosphere microbiome (leaf), anthosphere microbiome (flower), spermosphere microbiome (seed) and carposphere microbiome (fruit) (3). Microbes can even be transferred vertically through seeds (3). These microbes can further be characterized into two categories, epiphytes that stay on the surface of plant organs and endophytes that penetrate plant organs and form beneficial relationships with them (endosphere microbiome) (3). The plant microbiome primarily consists of bacteria that exert highly beneficial effects on plant development by direct or indirect mechanisms (24). They are termed as Plant Growth Promoting Bacteria (PGPB) (24). PGPB that are primarily found in the soil that surrounds the root surfaces are termed as Plant Growth Promoting Rhizobacteria (PGPR) (24). This review would focus on PGPR bacteria as in majority of the cases, beneficial effects are observed by PGPR living on or inside plant roots making them attractive for commercialization. The following section focuses on the specific functions that make PGPR widely popular for commercialization as microbials.

PGPR, that constitute the rhizosphere microbiome are of particular importance in crop protection due to the extensive plant-microbe symbiosis that takes place (3). This is because, the rhizosphere is an integral area of the plant ecosystem, that governs the chemistry of plant nutrient acquisitions (3). The host plant secretes exudates and signaling molecules that can recruit microbial counterparts from surrounding microbial reservoirs (3). In turn, the rhizosphere microorganisms produce vitamins, antibiotics, plant hormones, communication molecules, etc. that encourage plant growth and alleviate abiotic stress (3). The rhizosphere is also one of the major sites that contribute to entry of endophytes into plant roots (3). Though the exact mechanisms and modes of action through which rhizobacteria promote plant growth is not well understood, it is established that PGPR along with integrated nutrient management may be more effective for growth, yield and fertility status under sustainable agriculture (3,4,6). However, the microbiome of the plant is highly subjected to different environmental conditions such as pH, temperature, soil type, moisture and salinity (3). Additionally, microbial community is influenced by plant host factors and microbe-microbe interactions (3). It is due to this reason that different studies report great variation in plant microbiomes between species and within species themselves (3,4,6). This presents as a major challenge to companies working with microbials as they might find it difficult to produce a one-fits-all commercial product that is highly effective to a wide number of species. Companies currently produce a wide range of products with diverse PGPR as detailed in later sections.

VI. FUNCTIONS OF PGPR

Plant growth promoting rhizobacteria can be classified under three broad categories based on their beneficial effects in stimulating plant growth. The categories are biofertilizers, phytostimulators and biopesticides (25), as summarized in the table (table 1) below. It is important to note that many species of PGPR display all three or primarily two categories of beneficial effects (25). This fact makes many species of PGPR more appealing for commercialization.

TABLE 1
THE THREE FORMS OF PGPR CHARACTERIZED BASED ON THEIR BENEFICIAL EFFECTS. ADAPTED FROM P. N. BHATTACHARYYA ET AL., 2012 (25).

PGPR forms	Definition	Mechanism of action
Biofertilizer	Formulations that contain live microorganisms that aid plant growth through increased uptake of nutrients	Biological nitrogen fixation Utilization of insoluble phosphorous
Phytostimulator	Microorganisms that produce phytohormones such as IAA, gibberelins, cytokinins and ethylene	Production of phytohormones
Biopesticide	Microorganisms that promote plant growth through biocontrol of phytpathogens	Production of antibiotics, siderophores, HCN and hydrolytic enzymes Acquired and Induced systemic resistance

PGPR assert their positive effects on plants through two mechanisms- Directly or indirectly (26). Direct mechanisms is the ability of PGPR to provide plants with compounds that are directly produced by them or facilitate nutrient acquisition (26). Direct mechanisms involve the production of phytohormones, nitrogen fixation, increasing iron availability, phosphate solubilization, siderophores and ammonia production, etc (26). These functions are carried out by the production of specific enzymes that induce morphological and physiological changes in the plant host. Indirect mechanisms applies to the ability of PGPR to protect the crop from phytopathogens (26). Indirect mechanisms could involve the production of antibiotics, hydrogen cyanide (HCN), induced systemic resistance (ISR), and production of lytic enzymes such as chitinases, proteases, cellulases and lipases that can lyse the cell walls of many pathogenic fungi (26). Direct mechanisms of PGPR are termed as plant growth promotion effects. They are mainly characterized under biofertilizer and phytostimulator groups. Alternately, indirect mechanisms are termed as plant protection effects and characterized under the biopesticide group. Some of the mechanisms that are important to agriculture and commercialization are touched up on below. The main genera of PGPR that performs each function are highlighted in the next few sections.

6.1 Direct Mechanisms

6.1.1 Biological Nitrogen fixation

Nitrogen serves as the basic building block of plants, animals and microorganisms and is thus the most important nutrient required for plant growth and productivity (26). Nitrogen fixing PGPR fix molecular/atmospheric nitrogen to be further utilized by plants. They can do so in both symbiotic and free living systems. The main genera of symbiotic nitrogen fixers include: *Rhizobium, Achromobacter, Sinorhizobium, Azoarcus, Mesorhizobium, Frankia, Allorhizobium, Bradyrhizobium, Burkholderia, Azorhizobium,* and *Herbaspirillum* (26). Some of the important free living nitrogen fixers include: *Azoarcus sp., Herbaspirillum sp., Gluconacetobacterdiazotrophicus*, and *Azotobacter* (26). Even though they are highly diverse, all rhizobia establish symbiotic interactions with their host plant through highly conserved mechanisms that have been reviewed extensively (26).

Due to their importance, nitrogen fixing PGPR serve as the most important and recognizable examples of biofertility inoculants (6,26). Approximately 90 million metric tons of atmospheric nitrogen is fixed annually by legume crops that are grown globally on an estimated land of 250 million hectares (27). Several marketed products have been shown to affect consistent improvements in yields of legume crops in different studies (6). One study reported yield improvements averaging approximately 120 kg per hectare in *Rhizobia* inoculated soybeans (6, 28). Another study compares the commercial products based on their impact on soybean yields (28). These commercial products are all based on the *Bradyrhizobia* and other formulates or inoculates. Optimize® sold by Monsanto BioAg Alliance had outstanding result among all commercial products. It contains a mixture of *Bradyrhizobium* cells and lipochitooligosaccharide which is a molecule that enhances the soil microbial environment. In their study, they observe that seeds treated with Optimize® consistently show an increase in yield over untreated controls (6). In addition, they find that the use of bioinoculants on soybean crops consistently provides a 4:1 return on investment (6).

Out of the free-living nitrogen fixers, *Azospirillum* MicroAZ-STTM (TerraMax), Mazospirflo-2 (Soilgro) (31) *Azotobacter* Bio-NTM (Agriculture Solutions), and *Gluconacetobacter* have gained interest. They have been shown to increase the yield of various crops such as sunflower, carrot, oak, sugar beet, sugar cane, tomato, eggplant, pepper, cotton, wheat, and rice (6). In a survey of 20 years of global field trials, Okon et al., (1994) found that inoculation with several *Azospirillum* strains boosted crop yields by 5–30% in 60–70% of the trials (32). More recently, Diaz-Zorita et al. (2012) found that on-seed inoculation with *Azospirillum* enhanced wheat and maize yields by 244 kg ha-1 and 514 kg ha-1, respectively, in an expansive multi-year research (33). Except nitrogen fixation, some *Azospirillum* species can also produce plant growth-promoting chemicals in addition to nitrogen fixation, which may play a role in their mode of action (6). Other plant-beneficial features of non-leguminous nitrogen-fixing bacteria include heavy metal clean-up and increased plant tolerance to abiotic conditions like drought (6).

6.1.2 Phosphorous solubilization

Phosphorous is the least mobile nutrient and is the second most important nutrient in crop productivity, after nitrogen (6,26). It is found both in organic and inorganic forms in soil, but are not available to the plant (6). Phosphorus is thus usually substituted in agriculture through chemical fertilizers and manure (6). However, the long term sustainability of current phosphate sources are debated (6,26). Phosphorous solubilizing microorganisms offer a key solution to ensure efficient use of phosphorous in the environment (6). Soil microorganisms that release phosphate from organic and inorganic pools have been commercialized as products that successfully mobilize phosphate from soil's scarce sources, in turn reducing the use of chemical fertilizers (6,26).Some of the key genera that can mobilize phosphate include Pseudomonas spp., Agrobacterium spp., Azotobacter spp., Bacillus spp., Burkholderia spp., Enterobacter spp., Erwinia spp., Kushneria spp., Paenibacillus spp., Ralstonia spp., Rhizobium spp., Rhodococcus spp., Serratia spp., Bradyrhizobium spp., Salmonella spp., Sinomonas spp., and Thiobacillus spp (26).

Bacillus and Pseudomonas account for the most important bacterial genera as they have shown improved agricultural yields (34). They are present in commercial products such as Symbion-P® and JumpStart® respectively. Legget et al., in 2015 summarized maize yield responses to JumpStart® (containing Pseudomonas bilaiae) inoculation (6). Significant yield increases were observed in both small scale and large-scale plots (6). Interestingly however, for phosphorous solubilization, fungal species have been shown to have more solubilizing activity in comparison to PGPR species. Arbuscular mycorrhizal fungi (AMF) are well known fungal species of phosphate-solubilizing microorganisms (6). They may build a network of hyphae that interact with plant roots to increase nutrient delivery and provide plant protection (6). Some of the examples of AMF products include Mycormax® (JH Biotech), BEI (BioOrganicsTM), BioGrow Endo (Mycorrhizal Applications), and VAM (Microbesmart). Although, there seems to be an absence of highly effective commercial phosphate solubilizing inoculants due to lack of research into the mechanisms of phosphorous solubilization (6). In addition, plant and environment compatibility plays a huge role in the commercialization of phosphorous solubilizing products (6). Contrastingly, PGPR products for phosphorous solubilization seems to be more popular than AMF fungal species due to the ease of maintenance of bacteria over fungi in commercialization (6).

6.1.3 Production of phytohormones

Approximately 90% of PGPR are known to produce phytohormones (36). Phytohormones are chemical messengers that influence gene expression and transcription, cellular division, seed germination, flowering emergence, flower sex, leaf senescence, and fruit ripening (36). Plant growth and development are thus greatly regulated by phytohormones and can have an influence in plant phenotype, morphology and metabolism (36). The most well-known phytohormones are auxins, gibberellins, cytokinins, ethylene, and abscisic acid (36). These phytohormones play their own role in plant growth and regulations. For example, auxins and cytokinins are essential regulators of vascular development, root apical dominance, and lateral root initiation, as well as determining root architecture (36).

Bacteria belonging to the genera *Azospirillum, Pseudomonas, Xanthomonas, Rhizobium, Alcaligenes faecalis, Enterobacter cloacae, Serratia marcescens, Mycobacterium sp.,Burkholderia, Azotobacter, Bacillus cereus* and *Bradyrhizobium japonicum* have been shown to produce auxins which help in stimulating plant growth (36). Indole Acetic acid (IAA), which is the most physiologically active auxin in plants is known to be produced by almost 80% of all rhizosphere bacteria (36). In one study, they found that a majority of *Rhizobium* isolates from the field, produce IAA and may serve as PGPR in promoting growth of non-legume plants (36). In another study, IAA-producing strains of *Azospirillum brasilense* and *Bradyrhizobium japonicum* were shown to stimulate early growth promotion of seedlings of corn and soybean (36). In addition, auxins are said to be the most quantitatively abundant hormones produced by *Azospirillum* (37). These studies demonstrate the major influence phytohormone producing PGPR can have in crop improvement. PGPR have also been shown to produce over 30 growth promoting compounds of the cytokinin group, in addition to regulation of ethylene and abscisic acids (36). Most genera not only aid the plant by producing phytohormones, they also perform nitrogen fixation/phosphorous solubilization providing multiple benefits (6). The genera have already been commercialized as microbial inoculants and are detailed in table 2.

6.2 Indirect Mechanisms

6.2.1 Siderophores production

For the majority of microorganisms, iron is an important nutrient, and iron shortage can be fatal (36). Iron is present in the rhizosphere in small levels, and much of it is in a ferric form (Fe+++), which is poorly soluble and not readily accessible to microbes (36). Many PGPR in soils get past this difficulty by the creation of small peptidic molecules called siderophores.

These siderophores contain side chains and functional groups that provide ligands with high-affinity to which ferric ions can bind (36). The siderophores acts as a mechanism through which some PGPR can acquire iron and grow in the rhizosphere. Additionally, through this same mechanism, PGPR can effectively deliver iron to host plants encouraging plant development (26). For example, siderophores produced by Chryseobacterium spp. C138 when delivered to the root were effective in the supply of iron in tomato plant (38). In another study, siderophore producing *Pseudomonas* strain showed significant increase in germination and plant growth (39). In addition, bacterial siderophores can inhibit or reduce pathogen multiplication, by lowering the amount of iron accessible to a pathogen (36). Thus, siderophore producing microorganisms have been shown to have competitive advantage over other microorganisms in the rhizosphere (36). Siderophore production mechanism is an example where PGPR act as both fertilizer and pesticide.

6.2.2 Production of antibiotics and hydrolytic enzymes

The synthesis of one or more antibiotics is the main method used by PGPR to counteract the harmful effects of plant pathogens (26). Antibiotics are low molecular weight chemicals generated by PGPR (26). They effectively inhibit the development of other microbes by interfering with essential enzymes and metabolism, leading to biostatic and biocidal effects (26). Moreover, PGPR’s are able to produce one or more antibiotics, which can overcome the ability of the plant to acquire resistance to some specific antibiotics (26). This considerably improves their potential to function as effective antagonistic agents against pathogens as a whole (26). Bacillus spp and Pseudomonas spp. are known to produce many antibiotics including bacillomycin, surfactin, iturin, plipastin, pseudomonic acid, kanosamine, Rhamnolipids, among others (40). However, it was noted that antibiotics produced against one pathogen might not be as effective to control another pathogen on the same plant (26). This factor may limit the use of single spp. PGPR for use as biocides. Additionally, the effects of antibiotic-producing PGPR may be varied in field conditions, and the activity of biocontrol can be altered by methods of cultivation and formulation in the lab (26).

In addition to antibiotics, some PGPRs produce hydrogen cyanide and fungal cell wall degrading enzymes to inhibit fungal pathogens (26). In a study, they found chitinase, β-1,3-glucanase, and chitinolytic enzymes produced by some PGPR appear to inhibit the fungal pathogen *Rhizoctonia solani* (36). Additionally, HCN produced by rhizobacteria is an effective agent of biological weed control, as they have the ability to inhibit the electron transport chain and energy supply to the cell, ultimately leading to apoptosis (41). The production of HCN however is highly regulated, as large amounts could be toxic to the host plant itself (26). This needs to be considered when developing formulations for crop protection.

6.2.3 Induced systemic resistance

PGPR have also been shown to simulate the plants resistance mechanism to pathogens (26). Induced systemic resistance (ISR) allows the plant to have enhanced defensive capacity against infection by one or more pathogens (26). ISR uses plant hormones such as jasmonate and ethylene to stimulate resistance mechanisms in plants (26). Upon contact with pathogens, the plants are primed to defend themselves through effective ISR (26). Some PGPR directly regulate ethylene levels, while others have an effect through simulating ISR (36). Other PGPR can simulate ISR just by the presence of their individual microbial cell components such as lipopolysaccharides (LPS) and flagella, signal molecules, and some antifungal metabolites (36). For example, a study recorded that just the presence of Rhizobium spp. (through elicitation of isoflavonoid phytoalexin) has been associated with disease resistance in alfalfa and common bean. Additionally, ISR is not pathogen specific, thus making PGPR that activate ISR very potent in biocontrol (26).

In addition to the mechanisms mentioned above, PGPR have other effects such as competition in which PGPR compete for the uptake of nutrients with pathogens and predation where the PGPR use the pathogen as a food source. This is particularly observed in *Pseudomonas fluorescens*, when it colonizes of the hyphae of fungal pathogen *Fusarium oxysporum*, also inhibiting their spore germination. All these beneficial effects of PGPR work in tandem with each other and are highly advantageous compared to the few advantageous effects of chemical fertilizers/pesticides, which. Moreover, the use of agrochemicals have major effects on the natural rhizosphere microbiome, in turn harming this harmonious plant-microbe symbiosis.

The functions of PGPR and their direct and indirect effects on the host plant are summarized in the graphic below (figure 1).

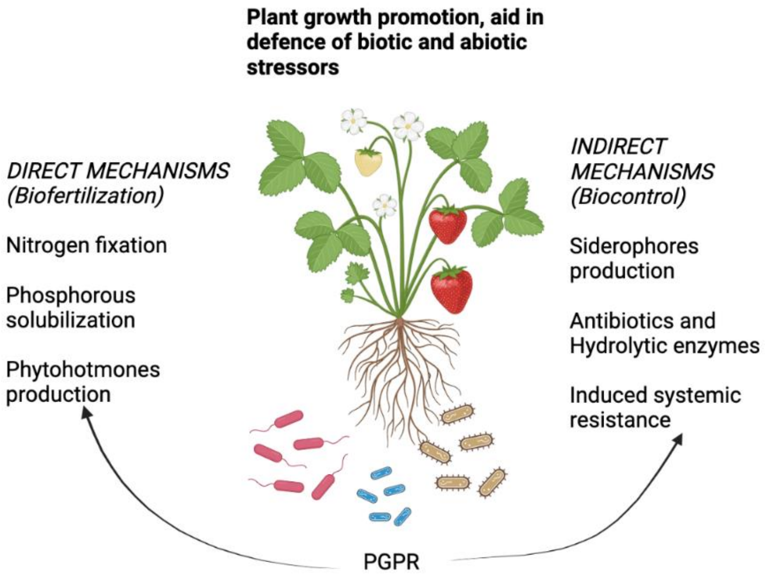

FIGURE 1: A summary of the beneficial effects of PGPR on plant growth.

VII. COMMERCIALIZED PGPR IN AGRICULTURE

According to literature surveyed, *Pseudomonas* and *Bacillus* were the most common bacterial genera identified in plants (26). In this section, we summarize the findings to obtain an overview of the main species of PGPR used in crop protection. The table details the particular crops on which the PGPR is currently being used and their significance. In addition, commercial products with the particular strain of species are highlighted to map which of the species are highly commercialized in comparison to other PGPR which are not yet in the market/new in the market. Ultimately, this section aims to evaluate if there's a correlation between commercialization, crops and function of species. Note: the species that are highlighted in the previous sections to have most effects/ significance are chosen.

TABLE 2
COMMON PGPR STRAINS THAT ARE USED IN AGRICULTURE. THE MAIN CROPS ON WHICH THE PGPR STRAIN IS USED AND THEIR MAIN FUNCTIONS ARE DETAILED. ADDITIONALLY, THE COMMERCIAL PRODUCTS THAT CONTAIN THE PARTICULAR PGPR AS THEIR ACTIVE INGREDIENT ARE MENTIONED

PGPR	Crop	Significance	Commercial Products	Reference
Azoarcus	Rice	Nitrogen fixation leading to increase yield of rice, tolerance to biotic and abiotic stresses		44,45, 46
Azorhizobium	Wheat	Nitrogen fixation		44,45, 46
Azospirillum	Wheat, Maize,Rice, Soybean Sugarcane	Nitrogen fixation	TwinN,(Australia) SymbionN, CALSPIRAL, Sadar Biofertilizers (India) Azo-green, Gmax,Nitromax	44,45
Azotobacter	Wheat, barley, oats, rice, sunflowers, maize, line, beetroot, tobacco, tea, coffee and coconuts, soybean, sugarcane	Nitrogen fixation	Azotobacterin, Ekophit (Southern and Eastern Russia), TwinN (Australia) Phylazonit-M (Hungary), SymbionN, CALZOTO, Sardar Biofertilizers (India); Dimargon1 (Colombia) Biogold, GmaxNitromax, Kisan Azotobacter, Astha azo, Sanjivini- N2, Nitrofix, BIO N MORE	42, 44, 45, 46

Bacillus	Potato, cucumber, pepper, wheat, eggplants, maize, peanuts, cauliflower, sugarcane, chickpea, soybean, tomato, cotton, barley, mungbean, grapes, apple	Auxin,cytokinin and gibberelin synthesis, potassium solubilization, induction of plant stress resistance, antibiosis effects	Bamil and Omug (Russia), Ekud (Russia), Pudret (Russia), Bactophosphin (Russia), Xin Sheng Li (Japan), Serenade (USA), SymbionN (India), Phylazonit-(M) (Hungary), UPMB (Malaysia), Probio96 (Iran) PIxPlus, Sonata ASO, Ballard, Epic, HiStick NT, Kodiak, Rhizoplus, Subtiex, Quantum 4000, Rhapsody, System 3, Companion, Bioyield, Rhizovital, Biotilis Gmax Phosphomax, KisanPSB, Astha PSB, UPAJ- K, eco-potash	42, 43, 44, 45, 46
Beijerinckia	Sugarcane	Nitrogen fixation		44,45
Burkholderia	Rice, Mint, Sugarcane, Chickpea, Apple		Blue Circle, Deny, Intercept	44,45
Chryseobacterium	Tomato	Siderophore production		44,45
Frankia	Alnus, tomato	Nitrogen fixation		44,45
Glomus *(Mycchoriza)*	Rice, cotton, soybean, corn, coffee, sorghum, sugarcane, tomato, banana	Nitrogen fixation	EcoMic (Cuba), Microfert (Cuba, Mexico), MYCOGOLD (Malaysia), Agri VAM, bio e rich	44,45, 46
Herbaspirillum	Sugar cane, bean, rice, sorghum	Nitrogen fixation		44,45
Mycobacterium	Maize	Induction of plant stress resistance		44,45
Paenibacillus	Lodgepole pine, black pepper	IAA synthesis, Potassium solubilization		44, 45
Phyllobacterium	Strawberries	Phosphate solubilization, siderophore production		44,45
Pseudomonas	Mung beans, wheat, cotton, maize, potato, tomato,	Chitinase and β-glucanases production, Induction of plant stress resistance, Antibiotic production, Siderophore production	CALMONAS (India), FOSFORINA (Cuba) BioJect, Spot-less BioJect, AtEze, Cedomon, Blight Ban A506, Conquer, Victus, Biosave (10,11, 100, 110, 1000), Proradix, BioPower Lanka, Gmax FYTON, Astha PF, SKS PF	42, 43, 44, 45, 46
Rhizobia	Legumes, peanuts	Nitrogen fixation, Induction of plant stress resistance, Hydrogen Cyanide production	R-Processing Seeds (Japan), Hyper-coating seeds (Japan)	44, 45
Rhizobium	Rice, pepper, tomato, lettuce, carrot, mung beans	Nitrogen fixation, IAA synthesis, ACC deaminase synthesis, phosphate solubilization, siderophore production	Mamezo (Japan), Nitrogin Gold (USA), SymbionN (India), CALOBIUM (India), Rizotorphin (Russia), Rhizobia, Sanjivini N1, Astharhizo	44, 45, 46
Bradyrhizobium	Pigeon pea Soybean,	Chitinase and B-glucanase production	Optimize® (USA), Vault® (USA), ExcalibreTM (USA), MycoGoldTM (USA)	44, 45
Sphingomonas	Tomato	Gibberelin synthesis		44, 45

Streptomyces	Indian lilac, soybeans, Cotton	Siderophore production	EM1R (Japan), EM Bokashi (Japan), Pixplus	44, 45

From table 2, it is apparent that *Bacillus* species is the most prevalent in this market. Many of the *Bacillus* strains (such as *B. cereus, B. pumilus, B. subtilis, B. amyloliquifaciens*) have desirable characteristics that make them particularly suitable as a product for commercialization (48). They are ubiquitous in soils, exhibit high thermal tolerance, and their rapid growth in liquid culture combined with ease of maintenance are advantageous (48). Additionally, they are considered to be a safe biological agent, having high potential in both the biocontrol and plant growth promotion sector (48). Nitrogen-fixing PGPR turn to be the second most highly commercialized strains. However, compared to Bacillus spp., nitrogen fixing PGPR are accounted for mostly in the plant growth promotion sector (6,26,43). Commercialization of Pseudomonas spp. appears to be not as expansive when compared to Bacillus spp. Although, they have similar desirable characteristics as Bacillus spp,, their low implementation rates could be because application of *Pseudomonas* is at infancy stage despite being extensively researched (6,43).

Overall, research and commercialization in general seems to be focussed on free-living rhizobacterial strains, especially to *Pseudomonas* and *Bacillus* (49). Unique associations are present between nonsymbiotic endophytic bacteria (such as *Frankia, Allorhizobium* and endophytic rhizobium) and host plants that leads to a more pronounced growth-enhancing effect on host plants (49), but not much information about them is found in literature.

VIII. CHALLENGES FACED BY MICROBIALS

The previous sections imply that there is significant evidence supporting the use of microbials in agriculture, with microbials outperforming chemicals. However, some concerns persist. These implementation barriers for microbials have been addressed by many authors, which are outlined by Gelernter et al., in 2005 and (23) and Ravensberg et al., in 2011 (20). The main reasons that were reported were: variable efficacy and quality of the products, their cost-performance level, cumbersome registration process, competition with agrochemicals, underestimation of the required investment and implementation time, overestimation of market size and market adoption rate, and lax collaborations between product developers and academic researchers in the industry (10, 20). Some of these challenges persist today. Thus, in this section, we would delve deeper into these challenges. The challenges identified can be broadly classified under three categories: Research and development, the global market and product registration. In this section of the review, we delve deeper into these categories to identify the bottlenecks that are preventing the widespread use of microbials.

8.1 Research and development

Much research has been dedicated to exploring the benefits of PGPR and the soil microbiome on host plants, but there is little evidence of translation of these effects in large scale applications (on-field) (6). Research by itself cannot stand alone and must go in hand with development and commercialization for effective use of the research. Several groups have criticized this issue in the applications of microbials in the agricultural market (20,49). Early on in 1997, Dent stated that the known information on microbial biological control was built up in a haphazard way (49). Individual scientists concentrated on their own studies and interests in comparison to the chemical pesticide business which collaborated with large R&D departments to produce crop protection products (49). More recently, Ravensberg noted that little had changed since then, and proposed that academic scientists and biopesticide developers must begin collaborating early in the product's research and commercialization phase (20).

Though research is more intensive since the first introduction of microbials in the market, important challenges remain. One of the main limiting factors preventing more widespread use of PGPB/PGPRs is their selectivity (13). Conventional agrochemicals often have a broad spectrum of effects on a variety of species (13). On the other hand, PGPB/PGPR tend to be quite focused (13). Previous sections (and table 2) have pointed out the plant host specificity of some PGPR. The PGPR *Azoarcus* for example is highly beneficial for rice crop, but is not found in the microbiome of other crops. For this reason, there are no commercial products existing with *Azoarcus*, albeit rice being a major commercial crop. Additionally, under field circumstances/ in complicated field environments where several organisms act concurrently, the use of PGPR might lead to varied quality and efficacy (13).

Second, the screening strategies of the microbial inoculants must be improvised. This is because the rhizosphere accounts for a diverse habitat for growth of microorganisms and community structure of plant roots (6,13). With this environment changing constantly due to stressors or other factors, it is important to involve differential quantitative and qualitative techniques to identify microbes (49, 50). Additionally, some PGPR such as Pseudomonas spp and *Bacillus subtilis* form biofilms and others are endophytic, which could make them tough to cultivate (50). Screening would lead to better characterization of species that have advantageous effects and overall plant-microbe symbiosis, in addition to discovery of novel species and better understanding of inter-species interactions. With recent developments in multi-omics technologies, other improved computational technologies that have been accordingly reviewed by JM barea, 2015 (51) and Godinez et al., 2021 (52), screening can be performed on a large scale and optimized.

The third issue that arises is the efficacy of the current microbial products on the market (4,6,7). Products have a maximum shelf-life of two-years, and this can be attributed to the active ingredient being live microorganisms (4,6,7). Moreover, in the case of biocontrol products, resistance of pathogens presents as a problem (26). Pathogens develop defense mechanisms that battle host defenses, sometimes with disease turning more aggressive compared to before (26). This is usually observed in microbial products with single species strains, and microbial products are rendered ineffective in such cases (4,6,7,26). Thus, the durability of the biocontrol products must be improved to contain either polymicrobial formulations and/or effective delivery systems (26, 36). The use of polymicrobial formulations will benefit the rhizosphere ecosystem and overall plant health, by combining different effects of the individual species (36). Commercial products containing multiple species of nitrogen-fixing microorganisms currently exist, however they are not commonly combined with other species such as *Bacillus* and *Pseudomonas* for biocontrol and biofertilization (6). Using such combinations would prove to be extremely beneficially for both the crops and the market, but this is an area of nascent understanding (36).

In addition to the issues mentioned above, efficacy of the microbial inoculants needs to be improved for overall research and development (4,6,7). The effectiveness of microbial plant protection solutions can be influenced by a variety of circumstances. Temperature, humidity, wetness (for example, in the soil or on leaf surfaces), plant growth stage, and other factors can influence microorganism behavior in a variety of ways (6). To integrate these, lab trials should be followed by wide-scale field trials to establish the complete effectiveness of the product (6,7).

Laboratory studies may offer information on the mode of action, susceptibility of target pests or hosts, including multiple life stages when applicable, dosage response behavior, and the impact of environmental, agronomic, and other conditions on the product (6,13). For proper dissemination of information to farmers and consumers, the overall conditions required for the microorganism(s) that make up a product's active ingredient to live, proliferate, colonize, or infect target species should be identified when possible, and advise given on the proposed product label if possible (6,13). Farmers could then make informed decisions in the use of microbials over agrochemicals (6,13). Moreover, polymicrobial formulations can be combined with effective delivery systems cold improve vastly the efficacy of microbials. The different delivery systems and their advantages/disadvantages are detailed in Bashan et al., 2015 (15).

8.2 Global PGPB/PGPR market

PGPB/PGPRs are commercialized as biofertilizers and biopesticide products (13). Biofertilizers and biopesticides are generally characterized by the product type, active ingredients, crop type, application and geography (13). In a market research report published by Transparency Market research, it was estimated that the Global Biofertilizers Market size was at USD 3,491.19 million in 2021 and expected to reach USD 3,842.76 million in 2022 (53). The market is projected to grow at a CAGR (compound annual growth rate) of 10.32% to reach USD 6,295.31 million by 2027 (53). For biopesticides, the global market in terms of revenues was estimated to be worth about 5.5 billion USD in 2022 (54). The market is expected to reach $9.6 billion by 2028, at a CAGR of 11.7% during the forecast period of 2021 to 2028 (54).

Over the projected period, North America is expected to lead the worldwide biopesticide/biofertilizer market in terms of demand (53,54). This demand can be owed to the growth of organic products, adoption of advanced irrigation systems and severe concerns towards the excessive use of chemical fertilizers (6,13). For the same forecast period, it is projected that the region of Latin America and Asia Pacific will be the most upward biofertilizer/biopesticide growth market (53,54). This trend is not predicted in Europe due to the long and cumbersome registration process and regulatory issues, even though the adoption of organic markets is high (6,13). Comparatively, in under-developed and developing countries such as India and Africa, slower growth could be attributed to high costs and general adoption of organic products in the market (14,26).

The current biofertilizer and biopesticide market however, represents just about 2.5-5% of the total agrochemicals market (13). Biofertilizers and biopesticides are mainly promoted as supplementary and complementary inputs and not as a replacement to chemicals (14,26). Farmers alternately choose chemical fertilizers as they remain cheap and easily accessible (6,13) As a result, agriculture still remains chemically intensive, and majority of farmers choose not to spend on additional inputs to reduce costs (6,13). Cumulatively, two key issues for the market can be distinguished: first, the biologicals programs have not yet succeeded in demonstrating the cost-effectiveness of the product to encourage governments to invest more and academics to carry out more research, which would accelerate the market development (7, 53). Second, land managers and farmers only see slow progress or initially no impact on yields and do not immediately see the financial benefits of microbials, compared to their usual pesticides that they see as reliable and predictable (53). To overcome this, programs and governmental initiative should be taken for proper dissemination of information to farmers and consumers (14,26). Furthermore, collaborations between private companies and small-scale industries, aimed at reducing costs can provide useful for these markets (6,7,13).

8.3 Challenges with product registration

Microbial product development is guided by regulatory frameworks and product registrations all around the world (55). The legislative framework for producing novel microbial products differs depending on the nation, the product's features, and its intended use (55). These regulations are strict as some PGPR have posed a risk to human health (7,55). This is because some microbial biocontrol agents have been reported toxic and pathogenic to non-target organisms (7,55). These national and international restrictions must be considered at every stage of the product development cycle, even the earliest stages, because regulations also specify the environments from where natural microorganisms can be harvested (55).

The registration process has long remained a hurdle in the microbials development process, and still appears to be so (20,55,56). The registration process in the European Union is complex for new active substances as they are categorized the same as chemical pesticides (55,56). This means that biopesticides would undergo the same regulatory measures as the registration of chemical pesticides (55,56). The guidelines currently used to evaluate biopesticides were originally developed for chemical pesticides and are mostly not appropriate for microorganisms (55,56). Furthermore, biofertilizers and biopesticides are grouped together as plant-protection products (PPP), which means that both go through the same process as for agrochemicals (55,56). This makes the process long and cumbersome, taking an additional 1.62 years (43%) on average, compared to procedures in USA (55,56). Furthermore, in the EU, the active substance is evaluated first, and only then begins the process for registration as a plant protection product (55,56). In contrast, the USA framework has a separate registration for biopesticides (56). The active substance and plant protection product are simultaneously evaluated, making the process less heterogenous, more flexible and less time (56). Also, the USA system uses ‘data waivers’, financial exemptions and conditional registrations to promote the registration process (55,56). This can be reflected in commercialization and in the market, giving a plausible reason as to why North America leads the biopesticide/biofertilizer market (53,54). In other developing countries such as India and Africa however, the registration process is more streamlined, but what remans challenging is the market (as discussed previously) (14,26).

This registration process itself might prove challenging for many companies and start-ups that want to bring a product into the market (6,26). With the recent implementation of Integrated Pest Management strategies in the EU, microbials are characterized as low risk substances (56). However, one requirement for low-risk substances, that is still to be elaborated, is that their half-life in the soil should be less than 60 days (56). This may be disadvantageous to researchers as the development of such a product will have to exclude some microbial pesticides such as rhizosphere competent antagonists of soil borne pathogens (56). Additionally, the long wait until the company can make profits could prove to be discouraging for the microbial market as a whole (6,14,26). Nevertheless, this year marks a huge change in the process for EU as they implement new regulations regarding biofertilizer products (55,56). These new harmonized regulations will come into force on 16 July 2022, and will differentiate biofertilizer products from biopesticides, allowing for a differentiated regulation process (55,56). The length of the registration period is also reportedly decreasing faster in the EU compared to the US (55,56).

IX. CONCLUSION AND FUTURE PERSPECTIVES

Microbials have long been used in agriculture, for their beneficial effects (6). They also present as an appealing, cost-effective alternative to agrochemicals (6,7). Microbes are simple target for companies working in organic ‘green’ agriculture and looking for a replacement for agrochemicals. Alternatively, developing interest in organic and sustainable farming in the past few years, has led to the characterization of more strains and species of bacteria that are beneficial in plant growth (24).

However, their potential applications in sustainable agriculture are still at the infancy stage. Fundamental challenges are present that bars the development of the whole microbials industry. In this review, we identify the challenges in each section of the industry to find areas of improvement. In the first section, we discuss the importance of microbials used in agriculture. We map out the important species of PGPR identified by research and compare to the active strains that are present in the current market. An interesting finding is that, although research has developed considerably, the market is still focused on *Bacillus, Pseudomonas* and nitrogen-fixing species. In the second section, the review focuses on detailing the key issues that are present for microbials in the sectors of research and development, market and registration. In this section, it becomes apparent that these three sectors are currently divided with various barriers within them.

For the future of microbials, it is logical that the sectors should not act independently to overcome these barriers. With more collaboration and dissemination of information amongst all participants (researchers, consumers, farmers), the potential of microbials would significantly improve. Recently, programs in the EU specify that member states have been encouraged to use rural development programs (funded under the Common Agricultural Policy) to provide financial incentives to farmers to start implementing microbials (56). With these improving regulations, discovery of new technologies and increasing adoption of sustainability worldwide, microbials can effectively replace agrochemicals in the near future. Overall, microbials now represent a significant division in the agriculture sector, and the future of microbials in sustainable agriculture remains attractive.

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