Tools and challenges to exploit microbial communities in agriculture

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A B S T R A C T

Plants contain diverse microbial communities. The associated microorganisms confer advantages to the host plant, which include growth promotion, nutrient absorption, stress tolerance, and pathogen and disease resistance. In this review, we explore how agriculture is implementing the use of microbial inoculants (single species or consortia) to improve crop yields, and discuss current strategies to study plant-associated microorganisms and how their diversity varies under unconventional agriculture. It is predicted that microbial inoculation will continue to be used in agriculture.

Sustainable agriculture and soil microorganisms

Most countries depend, directly or indirectly, on agriculture for their economy (Gupta et al., 2018). However, agriculture has changed since the end of World War II with an intensive use of new technologies such as mechanization, and the excessive use of chemical fertilizers and pesticides (Brodt et al., 2011). This has resulted in increased yields for important crops like corn (157%), rice (109%) (Díaz et al., 2020), and wheat (25%) ( Lobell et al., 2005). Although these developments have the positive effect of higher yields, they also generated significant environmental costs and damage, including the depletion of the soil surface layer due to mechanical tillage, the contamination of groundwater, air pollution, and a decrease in the diversity of beneficial microorganisms in the soil (Brodt et al., 2011; Prashar and Shah S. 2016; Hussain et al., 2009; Mishra Babasaheb Bhimrao et al., 2017). In recent years, the development of new strategies to mitigate the effects caused by intensive agriculture have become more relevant. These strategies are based on crop rotation, intercropping, reduced mechanical tillage, sowing of cover crops and reducing the dependence on chemical fertilizers by the use of biofertilizers constituted by soil microorganisms (Arora, 2019; Rosenzweig and Tubiello, 2007). Biofertilizers may provide nutrients to plants and improve the performance of crops (Sevilla & Mingorance 2015; Mukhtar et al., 2017). They can regulate soil microbial communities and thereby restore the soil microbiome and improve crop yields, and they can act as antagonists of phytopathogens in the soil (Kang et al., 2013; Dong et al., 2018; Santos, Nogueira and Hungria, 2019).

However, the success of biofertilizers depends on edaphic and environmental conditions, crop genotype, autochthonous microbiota, and some practical aspects such as accessibility to these products (Mitter et al., 2021; Da Costa et al., 2014). Organic matter content and soil pH may determine the effect of biofertilizers (Montiel-Rozas et al., 2017; Davison et al., 2021). The genotype of the plant is related to the effectiveness of biofertilizers, it is known that plants can directly or indirectly alter the habitat of the rhizosphere through rhizodeposits and changes in root architecture (Saleem et al., 2018). Another aspect to consider is the autochthonous microbiota since the presence of this can represent an antagonistic effect for biofertilizers (Debnath et al., 2019, Trejo-Aguilar et al., 2021); the autochthonous microbiota is more adapted to the rhizosphere environment and therefore better adapted to the environmental conditions and genotype of host plants. Islam et al., 2021 reported the survival of arbuscular mycorrhizal fungi (AMF) during three seasons (2001–2013), using pyro-sequencing and showed that at the end of the third season the biofertilizer was still present and had caused some alterations in the abundance and diversity of indigenous AMF. On the other hand, there are cases where the microorganisms inoculated in the field do not persist; for example, the inoculation of a consortium dominated by lactobacilli mixed with leachates of cow manure in corn
plants. After 130 days, lactobacilli had decreased relative abundance, and were displaced by the autochthonous microbiota (Afanador-Barajas et al., 2021).

Inoculants and consortia used in sustainable agriculture

It is estimated that in one gram of soil there are approximately 10^{10} bacterial cells and a diversity of species between 10^3 and 10^8 (Raynaud and Nunan, 2014). These microorganisms constitute a dynamic community, and because plants provide an environment that favors microbial growth a diversity of microorganisms interact with plants. Part of this interaction is mediated by the release of specific compounds either by the roots of the plant, which allows the development of specific microbial communities, or by bacteria, fungi or archaea (Kaur and Purewall, 2019). Biofertilizers increase plant resistance to both biotic and abiotic stress (Singh et al., 2019). An additional benefit of the use of microorganisms is the bioremediation of polluted soils, as they can sequester heavy metals, decompose pollutants, and recycle nutrients (Verginer et al., 2018). A widely used fungal biofertilizer, the arbuscular mycorrhizal fungi (AMF) may be used for this purpose (Chen & Glover, 2016) as their external hyphae contribute to plant uptake of less mobile nutrients and alleviate heavy metal toxicity (Ferrol et al., 2016), in addition to protecting the roots of the plants from direct contact with contaminants (Wenzel, 2009).

Among the main genera used as inoculants on crops of interest are Rhizobium, Azospirillum and Bacillus (Elavarasi et al., 2020). Rhizobia are diazotrophic bacteria that associate with legumes and carry out biological nitrogen fixation under symbiotic conditions (Liu et al., 2018; Q. Wang et al., 2018). The use of rhizobia in crops is of agricultural interest, as it is considered safe and the interaction favors the growth of the plant through nitrogen fixation, solubilization of phosphates, inhibition of pathogens and resistance against some type of stress. Bertrand et al. (2015) evaluated the growth of alfalfa plants inoculated with a Rhizobium strain under soil salinity conditions (80 mM NaCl) and found that uninoculated plants had, decreased biomass weight, root and shoot weight, while those inoculated with Rhizobium increased their biomass up to 20%. Rice growth is also favored when it is inoculated by Rhizobium, which causes increased root growth, number of shoots, photosynthetic rate and an increase in grain yield up to 69% (Chi et al., 2005).

Azospirillum is known for its growth promoting effect in plants, with the release of secondary compounds such as amino acids, indole acetic acid, cytokines, gibberellins, and polyamines favoring root growth and, consequently, improved absorption of water and nutrients by the plants (Thuler et al., 2003). In addition to these benefits, Azospirillum has nitrogenase activity responsible for nitrogen fixation in free-living conditions for species of non-legume plants (Zeffa et al., 2019; Bashan and De-Bashan 2010). Due to these characteristics, Azospirillum is used as an inoculum in sugar cane crops where the average length and diameter of the stem, and the amount of sugar were observed to increase (Lopes et al., 2012). In corn, Azospirillum leads to increased plant growth, nitrogen concentration and foliar phosphorus, and higher yield (Coelho et al., 2020). Similar effects were also observed in different corn genotypes grown under nitrogen deficiency conditions (Zeffa et al., 2019).

Bacillus produces lipopeptides, lytic enzymes and endotoxins that exert a biological control against pathogens that affect corn, wheat and fruit trees (Fernanda Villarreal-Delgado et al., 2018; Jha et al., 2016; Miljusevic-Maric et al., 2018; Zalis-Kolsi et al., 2016). Bacillus subtilis has also been reported to produce hormones and solubilize phosphates (Luna Bentos Lobo et al., 2019). Bacillus xeniamensis produces indole acetic acid, ACC deaminase, and solubilizes phosphorus, thus increasing plant biomass in sugar cane; in addition to its anti-fungal pathogen effect (Amma et al., 2020). Breedt et al. (2017) found an increase in the yield of corn plants (34%) using as inoculum Lysinibacillus sphaericus (a bacterium phylogenetically close to the Bacillus genus). This bacterium also expresses nitrogenase and produces indole acetic acid.

Enterobacter cloacae in corn improves plant growth under drought conditions, helping the crop to increase length (24%) and biomass, and the increase in corn grain yield (60%). These benefits are due to the production of ACC deaminase, which reduces the adverse effects of ethylene stress (Danish et al., 2020). Verma et al. (2016) also reported that Enterobacter cloacae, due to its ability to solubilize phosphate, favored longitudinal growth and increased biomass in the corn plants.

Though inoculants composed of a single bacterium favor the growth of crops, in soil, microorganisms are found in communities, therefore to mimic soil communities some new inoculants are composed of Multiple species. Pereira et al. (2020) showed that co-inoculation with Bacillus subtilis and Azospirillum brasilense promoted the absorption of phosphorus and increased corn grain yield 54% as compared to single strain inoculation (Pereira et al., 2020). The co-inoculation of Bacillus subtilis and Azospirillum brasilense also improved shoot production (38%) and reduced phosphorus fertilization up to 75% in sugarcane (Rosa et al., 2015). The co-inoculation of Rhizobia with Bacillus megaterium generated a synergistic effect on bean growth and favored nitrogen fixation (31%) (Korir et al., 2017). Inoculants with more than one species have been formulated (Fukami et al., 2018; Chibeba et al., 2020; Zeffa et al., 2020). Wang, Chen and Fu, 2019 tested dual inoculants of Bacillus megaterium with Pseudomonas fluorescens and Azotobacter chroococcum with Azospirillum brasilense and observed that the availability of nitrogen and phosphorus in the soil was increased, as was the growth of Cyclocarya paliurus plants compared to uninoculated controls. Barbosa et al. (2021) observed that co-inoculated Azospirillum brasilense and Bradyrhizobium spp. on soybean plants in soils in Brazil, increased root biomass up to 11%.

Gomez-Godinez et al., 2019 reported that a multi-species inocula composed of five bacteria (Rhizobium phaseoli Ch24–10, Azospirillum brasilense sp.7, Sinorhizobium americanum CFNE1156, Methylobacterium extorquens AM1 and Bacillus amyloliquefaciens CCGE203), increased corn plant size and biomass up to 113% compared to non-inoculated plants, and nitrogen fixation 215%. There have been changes in the taxonomic assignments, as some strains considered as B. subtilis were reclassified as B. amyloliquefaciens, and later as B. velezensis, thus different biocontrol agents are now recognized as B. velezensis. The commercial product Fungifree AB that contains antifungal bacilli contains B. velezensis (Balderas-Ruiz et al., 2020) and the Bacillus strain used successfully in a multispecies inoculum in corn roots (Gomez-Godinez et al., 2019) would also correspond to B. velezensis.

Among biofertilizers, the co-inoculation of PGPRs and arbuscular mycorrhizal fungi (AMF), is considered an additive strategy. However, Marulanda-Aguirre et al. (2008), found that the co-inoculation of Bacillus megaterium with native or commercial AMF species had contrasting effects on the development of the plant. When inoculated with a native AMF consortium, B. megaterium caused a reduction of the extra-radical mycelium in the AMF and the beneficial effect in the mycorrhizal association with the plant was not noticeable. In contrast, coinoculation with a commercial mycorrhizal species produced an additive effect that resulted in better plant growth.

The use of a single AMF species compared to AMF consortia also showed differences when applied to coffee plantlets, where consortia significantly increased plant growth. Hernandez-Acosta et al. (2020) demonstrated that in different coffee varieties the inoculation of a mycorrhizal consortium had a greater effect on plant growth than an individual inoculation of Rhizosphagus aggregatus. Furthermore, each variety of coffee responded differently to each inoculant, indicating that biodiversity of biofertilizers in relation to the plant genotype should be considered.

When used in the field, mycorrhizal inoculation can sustain fruit yield and quality in tomatoes, with 13% less fertilizer doses and optimized irrigation as compared with the farmers management (Biel et al., 2021). In a metaanalysis, Zhang et al. (2018) found that among seven
important cereal crops, the inoculation with AM fungi increased at least 16% the yield, regardless of the fertilization dose, thus contributing to a sustainable agriculture without compromising grain yield.

Knowledge on bacteria-plant interactions has provided distinct strategies to modify bacteria genetically and differently some modified bacteria have been found to be superior to reference strains. Examples include strains modified to excrete ammonium or having up-regulated nif genes (Geddes et al., 2015; Ambrosio et al., 2017; Bageshwar et al., 2017). However, the large diversity of available natural strains has not been fully tested and there may be cases where the latter may best to promote plant growth. In countries where the use of modified bacteria is restricted, the screening of native isolates is desirable.

Culture-dependent and independent approaches to study soil microorganisms

Studies of plant-associated microorganisms have used culture-dependent techniques although many of the microorganisms present in plants may not be cultivable in vitro. There are different culture-dependent techniques used to quickly characterize bacteria such as Gram, Ziehl-Neelsen and Schaeffer and Fulton stains (Beveridge, 2001), biochemical test kits (Figdor et al., 2008), carbon source utilization profiles (Stefanowicz, 2006), fatty and protein acid analysis (Zelles L. 1999; Salmonová and Bunečková, 2017), and others described in Bergéy’s systematic bacteriology manual (Holt et al., 2013). The culture-dependent characterization is usually phenotypic and biochemical, for which gender is identified, but not species (Figdor et al., 2008; Escobor-Zepeda et al., 2015). Further, a sequence-based analysis may define the isolate species.

The use of culture-independent high-throughput sequencing has been a tool that has unveiled the vast diversity of these plant-associated microorganisms (Berg et al., 2016; Hu et al., 2020). It must be considered that these techniques are descriptive, and we can obtain the structure, diversity, and identification up to the species, however, we cannot obtain the isolated microorganisms for use. The omics sciences have allowed the identification and characterization of genes involved in plant-microbiome interactions. Genes involved in bacterial colonization of plants may be involved in the adaptation and in the interaction of the communities that make up the microbiome (Dustogeer et al., 2020).

With high-throughput sequencing, we can study soil microorganisms using two approaches: 1) Sequencing of marker genes (metabarcoding) or 2) whole-genome sequencing (WGS). For the metabarcoding strategy, primers directed to the gene of interest, generally the 16S rRNA gene for bacteria or archaea and the intergenic sequences of the 18S rRNA gene (ITS) for fungi, are used. These methods are fast and allow a global vision of the organisms present in a microbial community (Knight et al., 2018; Luz Calle, 2019).

The second approach is a method that allows the identification of all the genomes present in a community. This means that the total DNA of the sample is sequenced and the genomes of each individual present in the community are bioinformatically separated (binning) (Wang et al., 2019), allowing a taxonomic and functional view of the most abundant members of the sequenced ecosystem (Fig. 1) (Knight et al., 2018; Luz Calle, 2019). Metabarcoding and metagenomic studies have made it possible to capture sequences of “rare” organisms, most of which are difficult to identify by traditional culture or cloning techniques. Obligate symbionts such as AMF or AMF associated bacteria, may not be culturable in media and are dependent on the hosts. Efforts to cultivate AMF fungal species in vitro have succeeded with only a dozen species compared to the more than 300 AMF species reported (Redecker et al., 2013). Using metagenomics in AMF represents a challenge, as their symbiotic state usually limits the amount of DNA available for this genetic approach (Bhargava et al., 2019). A recent study that combines cell sorting in AMF structures with sequencing may allow a more refined study (Montoliu-Nerin et al., 2020).

Albanese and Donati in 2017 proposed a method called StrainEst, based on the use of profiles of single nucleotide variants of available genomes to determine the number and identity of coexisting strains and / or species in metagenomic samples. This approach could help quantify and identify the different specific strains present in the rhizosphere. On the other hand, unique CRISPRs can be used as molecular markers for the identification of microorganisms (Sorek, Kunin and Hugenholtz, 2008). CRISPRs are present in approximately 50% of bacterial genomes (Westra et al., 2019), including some growth-promoting bacteria (Horvath and Barrangou, 2010) such as Azospirillum, Azotobacter, Nitrobacter, Nitrosomonas, Xanthomonas, Sarcina and Frankia. Their CRISPR loci are deposited in databases, so these could be used for the typing and monitoring of microorganisms in the rhizosphere (Rilling et al., 2019; McGhee and Sundin (2012)) compared 85 strains of Erwinia amylovora and managed to identify and differentiate strains with CRISPRs; this strategy could be used to develop molecular markers to monitor microorganisms in the soil, roots, and rhizosphere.

Omic technologies, StrainEst, CRISPR and cell sorting help to understand in a deeper way the structure of microbial communities associated with plants. In addition, these tools have revealed new functions of the plant microbiome, and interactions with the ecosystem, and how these effects, together, favor the health of the soil, plants, and the agroecosystem (Berg et al., 2017; Jansson and Baker, 2016).

Using culture-dependent techniques, we may isolate and identify...
microorganisms that can be experimentally tested for their effects on plants. Wang et al. (2020) described the bacterial diversity associated with marine sediments, of the 478 OTUs reported with the independent culture methods, 27 OTUs were present in the bacteria detected by culturable methods. Cultured strains belonging to *Aeromicrobium*, *Janibacter*, *Maribacter*, *Nesterenkonia* and three other genera were not detected by massive sequencing. In maize, the bacterial diversity of the rhizosphere was evaluated with the dependent and culture-independent approaches, finding that 30% of the sequences do not show similarities with the known taxa and were considered as new sequences. Using culture techniques, some bacteria with growth promoting capacity such as *Pseudomonas*, *Bacillus* and *Azospirillum* were identified; the latter was not detected by culture-independent techniques (Qaisrani et al., 2019). These results together help us to understand how, through culture-dependent techniques, we can isolate and identify microorganisms with some potential in growth promoting. A culture dependent approach is certainly complementary to the omics approach.

**Microbial diversity in different agricultural systems**

Most of the strategies, management and culture practices in agriculture influence the diversity and composition of the microbial communities that make up the soils. There is evidence that in crops managed organically or with conservation tillage, the microbial communities are enriched and thereby improve the physical and chemical characteristics of the soil, pest biocontrol and community biodiversity (Crowder et al., 2010; Hartmann et al., 2015; Liao et al., 2018). Schmid et al. (2011) showed a difference in vine crops under two different management systems, conventional and organic, finding that in the latter there were statistically significant differences in the structure and function of the associated microorganisms that favored the inhibition of pathogenic organisms such as *Botrytis cinerea*.

Souza et al. (2013) reported a metagenomic analysis where they compared a conventional tillage system and a no-tillage system, identifying the former as having a higher abundance of nitrogen-fixing rhizobial species. A large abundance of *Gemmatimonadetes*, *Xanthomonadales* and *Acidobacteria* was found in the Argentinean Pampa soils under a conventional tillage system, while a higher abundance of *Nitrospirae* was present in the no-tillage system. It was suggested that the community in conventional tillage might be adapted to a large use of high input nitrogen fertilizer that was positively related to the abundance of COGs involved with glutamate synthase (GOGAT) and the tricarboxylic acid cycle (TCA), such as citrate synthase and succinate dehydrogenase (Carbonetto et al., 2014). In another report, the change in diversity under different tillage systems and crop residue management was evaluated, with the finding that bacterial communities fluctuated depending on the tillage system to which they were subjected (Romero-Salas et al., 2021).

Lin et al. (2019) evaluated the effects of the use of chemical and organic fertilizers in tea crops and found that tea cultivars with organic fertilization had higher amino acid content, and lower Pb, Cd and Cu content compared to conventional management. Soil microbiota under the organic agroecosystems showed an increased relative abundance of *Burkholderiales*, *Myxococcales*, *Streptomycetales* Nitrospirales, *Ktedonobacterales*, *Acidobacteriales*, *Gemmatimonadales* and *Solibacterales*. All these reports indicated a correlation between agricultural practices and the microbiome, not only on diversity, but also on its functional capabilities and metabolism. Agriculture today faces a challenge, which is to increase crop production and at the same time avoid soil degradation and preserve diversity and ecosystems.
Perspectives

Microorganisms have been used in agriculture for a long time for their ability to promote plant growth, exert biological control or mitigate the effects caused by different types of stress. However, most of these inoculants show varying effects in field conditions that differed with climate, soil type and environmental conditions. In many cases, microorganisms used as inoculants are isolated from a different environment, which makes them less apt to compete with indigenous microorganisms. The variable performance of microbial inoculants suggests that their success depends on interactions with the plant, with the environment, and with the community of microorganisms present in the soils of Fig. 2.

To make the best use of microbial inoculants, it is essential to recognize that they are part of complex communities and are interacting with their environment and other organisms all the time.

In recent years, communities composed of beneficial microorganisms have been developed, which can be considered as communities or synthetic inoculants, to improve yields of the plants of interest (Gómez-Godínez et al., 2019). Evaluation of the adaptation, synergism and genes involved in plant-bacteria interactions has been done through the study of synthetic communities (Biswas et al., 2017).

It is not probable that universal microbial inoculants can be generated. The different approaches that range from the description of diversity, functionality and in silico metabolic networks, we will be able to explore different strategies to select members of synthetic inoculants that are better adapted to different types of soil, cultivation, and type of tillage. Synthetic microbial inoculants could provide the opportunity to validate predictions and correlations between members of indigenous communities, taking advantage of existing resources Fig. 2. However, a main challenge remains, in most cases biofertilizers do not provide the amounts needed to reach maximum yields that are obtained with inoculated fertilizer and besides, biofertilizer effects are not universal in all crops meaning that search of strains and their combinations are still unknown which plant growth strategies, with biofertilizers or microbial inoculants. The variable performance of microbial inoculants promotes plant growth—A critical assessment. Adv. Agron. 108, 77–136.

Decloration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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