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The Microbiome of Cassava (*Manihot esculanta*)

Andri Frediansyah

Abstract

The plant microbiome, like the plant, influences the processes that lead to plant development, health, and crop productivity. Cassava is a perennial herbaceous plant native to South America that has been cultivated for centuries as a staple food throughout the world. Not only is cassava a good source of carbohydrates, but it also has a high tolerance for a variety of phenotypic conditions, and the majority of cassava plants are susceptible to a variety of diseases. Thus, using cassava as a model, this chapter discusses the plant microbiome. We discuss the structure and function of the microbiome, as well as the technique for studying microbiomes. Additionally, we conducted a systematic review of references pertaining to the microbiome of the cassava plant using cultivation-dependent or cultivation-independent methods. Numerous significant genera of bacteria and fungi are found in cassava's phyllosphere and rhizosphere, including groups of gram-negative bacteria, gram-positive Actinobacteria, and gram-positive non Actinobacteria. Additionally, we identified critical organisms in the phyllosphere and rhizosphere. Cassava endophytes also produce antifungal secondary metabolites such as pumilacidins and surfactin. The investigation of their phenotypes and interactions with the cassava plant will aid in increasing productivity.

Keywords: cassava microbiome, metagenomic, plant microbiome, staple crop, phyllosphere, rhizosphere

1. Introduction

The microbiome was defined for the first time as the ecological niche within the human body where symbionts, pathogens, and commensal or neutral microorganisms coexist [1]. It is then widely used in a variety of habitats infested with microorganisms, including plants and their microbes. As with the plant itself, the plant microbiome influences the various processes that contribute to plant development, health, and crop productivity [2]. These connections have an effect on both nutrient absorption and susceptibility to biotic and abiotic stress [3]. Furthermore, factors such as regional landscape, plant species and cultivars, genotypes, soil, soil-borne microorganisms, climate and other environmental factors, farming management practices, and crop safety all influence the microbiome's dynamic and distribution [4–6]. Moreover, microbes associated with plants colonized both the plant's surface and internal tissue. They are frequently referred to as the plant's second genome due to their presence in the inner plant bodies as well [7]. Additionally, the complexity of nearly all plant microbiomes including its rhizosphere is still unknown [8].

Additionally, there is still a knowledge gap regarding plant-colonizing microbes, their interactions, and the microbiome's structure.

Cassava (*Manihot esculenta* Crantz) is an herbaceous perennial plant native to South America that is a member of the *Euphorbiaceae* family [9]. It is widely grown in tropical and subtropical regions [10]. Cassava was grown on a global scale of up to 201 million hectares in 2017, with Africa accounting for more than 60% of the total [11]. Furthermore, Nigeria was the largest producer of cassava, followed by Thailand and Indonesia [9, 11]. Cassava's tuberous roots contain an unexpected amount of starch, making it an extremely valuable food source, particularly in developing countries. As a result, cassava has developed into a staple food for roughly 800 million people worldwide [11]. Crop management and fertilization [12], food process development and fermentation [9, 13–17], component functional status [18], cassava disease [19, 20], and raw material and product quality control [21, 22], are just a few of the cassava-related studies published worldwide.

Cassava plants, like other plants, support a diverse range of microorganisms and plant-microbial interactions that enable the crop to perform a variety of task [23]. As illustrated in **Figure 1**, the cassava microbiome is distributed throughout the plant's body, including the portion of the upper and lower leaf surface (phyllosphere) that contains stems (caulosphere) and leaves (phylloplane), as well as the portion of the bellow grounds that contains roots and a trace of associated soil (rhizosphere). Within compartments, fungal and bacterial (and, to a lesser extent, archaeal) communities can be classified as epiphytes, which colonize the exterior surface of plant tissues, and endophytes, which penetrate the outermost plant cell layer (epidermis) and colonize the internal intercellular and intracellular sections of plant tissues.

Figure 1.
The distribution of microbiome in cassava plant.

Cassava is not only a good source of carbohydrates, but it also has a high tolerance for a variety of phenotypic conditions, including heat, nutrient deficiency, and drought [24, 25]. Additionally, the majority of cassava plants are susceptible to a variety of diseases, including cassava brown streak disease, cassava mosaic disease, and cassava bacterial blight [26–28]. As a result, our understanding of these correlations with the vastness of the microbiome is still limited at the moment. Thus, in this chapter, we will use cassava as a model plant to investigate its microbiome. Each cassava plant compartment is thoroughly examined. Additionally, we discuss the microbiome's structure and function, as well as the data collection methods used. Finally, we investigate the possibility of manipulating the microbiome to increase cassava crop productivity.

2. The technique to study cassava microbiome

There are two approaches to studying the cassava microbiome: cultivation-dependent and cultivation-independent approaches. Historically, the cultivation-dependent method was used to evaluate microbial communities. This strategy is based on the technique of microbial isolation. However, this is debatable given that only 1% of microbes can be cultured in the laboratory [29]. This is because a variety of factors affect microbes' cultivability, including nutrients, oxygen levels, temperature, salinity, pH, and growth factor [30, 31]. This technique has numerous advantages, including the ability to cultivate culturable microbes, the ability to quantify the cell, and the ability to identify viable cells in samples. Therefore, as consequence, using this approach results in a low level of taxonomic diversity, contamination, the requirement of time and resources, and the reliance on phenotypic biochemical characterization.

Without cultivating the bacteria, a molecular technique utilizing unprecedented amounts of 16S RNA or ITS data, such as denaturing and temperature gradient gel electrophoresis [32] and single-strand conformation polymorphism [33]. Additionally, polymorphisms in the terminal restriction fragment length [34], restriction analysis of amplified ribosomal DNA [35], random amplified polymorphic DNA [36], and sequencing of SSU ribosomal DNA [37], can be used to determine the microbial composition of the sample.

Moreover, recent advances in high-throughput sequencing, combined with a variety of omics techniques [38–41], have enabled researchers to gain a new level of understanding of the microbiome's structure and dynamics, as well as host-microbiome interactions. It also can provide a wealth of information about the microbial partners of a plant, including their identity and relative abundance [42, 43]. Therefore, employing this cultivation-independent approach, using sequencing technology, may result in an avalanche of data, which must be mitigated by using an experimental design and technique that are appropriate for the scientific question at hand [44–46]. It is critical to have a thorough understanding of the various types of biases and errors that can occur when selecting the system.

In plant microbiome research, including cassava, high-throughput sequencing of marker gene amplicons is increasingly being used to elucidate the structure, organization, and spatial distribution of microbial communities [5, 47–49]. Amplicon sequencing has the distinct advantage of being able to target specific microbe classes or even functional genes. Although the high specificity of amplicon sequencing enables it to positively classify unusual organisms, it is susceptible to contamination due to its sensitive nature [50]. Thus, any experiment involving a significant amount of amplicon sequencing should include both positive and negative controls [51].

When it comes to confirming the existence of rare organisms, shotgun metagenomics is less robust than amplicon sequencing [52–54]. The abundances measured, on the other hand, are less skewed, and the data can be binned into draft genome sequences [54–56]. These enable us to connect taxonomic identity to essential plant functions like nitrogen fixation, or to determine whether symbionts can communicate with plants via secretion systems or effectors. Metagenomic approaches also can supplement other high-throughput molecular methods such as transcriptomics, proteomics, and metabolomics [57–59].

In general, these techniques provide access to a microbial genetic pool that cultivation-dependent techniques do not provide, which means that microbial isolates do not need to be cultured because sequences are generated directly from environmental samples. High specificity and the ability to freeze samples for later use are also advantages. However, we were unable to obtain colonies for further research. Furthermore, there is a high risk of contamination with this technique, and the researchers are unable to distinguish between living and dead cells. Last but not least, the method is dependent on a well-designed primer plate, precise sequence identification, and a high-quality cell lysis process.

3. The phyllosphere and its microbiome

The phyllosphere is the first compartment in the microbiome of the cassava plant. This compartment is the visible portion of the leaf surface on both the upper and lower leaf surfaces [60]. Cover only the area above the ground, however. Microbial cells can colonize aerial plant surfaces such as leaves (phylloplane) and stems in this environment (caulosphere) [61]. Leaves may be one of the largest microbial habitats on the planet, with an estimated global terrestrial leaf surface area of 10^8 km^2 [62]. Along with bacteria, filamentous fungi, archaea, viruses, yeast, bryophytes, lichens, protozoa, and nematodes thrive in this environment. Bacteria, on the other hand, have been found to be the most abundant cell type in the phyllosphere, with up to $10^7 \text{ cells cm}^{-2}$ of leaf tissues present [63]. Another type of microorganism, filamentous fungi, appears to be more prevalent [63]. For all of these leaves' living things, water and food are scarce resources.

Special consideration will be given to endophytes when it comes to the cassava microbial community. Endophytic microorganisms are microorganisms that live inside the tissues of plants without harming the host [64]. The majority of endophytes spread systemically via the xylem to various plant compartments such as the stem, leaves, and fruits. They maintain the plant's viability throughout or part of its life cycle by colonizing the internal leaf tissues (endophyllosphere) and internal plant reproductive tissue [65]. Due to the fact that they live within the tissue, their nutritional requirements are also reduced [66]. As consequences, they multiply and grow rapidly within the plant tissue. They defend themselves by producing toxins and enzymes that aid them in colonizing the plant and competing with other microorganisms. Additionally, several of them produce beneficial secondary metabolites such as antibiotics, antifungals, anti-inflammatory agents, and biological control agents as part of the host's development and physiological process [67].

Melo, Fiore [68] successfully cultured several endophytes bacteria from the cassava phyllosphere using a cultivation-dependent approach. They were able to grow bacteria from cassava stems (23 strains) and leaves (17 strains). The 16S rRNA coupled with fatty acid methyl ester (FAME) assay could only be used to examine a small number of bacteria. *Bacillus* was found to be the most prevalent bacteria in this study [68]. *Bacillus anthracis*, *Bacillus pumilus*, *Brachybacterium paraconglomeratum*, and *Brevibacillus brevis* were discovered in the cassava stem, as well as

gram-negative bacteria *Enterobacter aerogenes*, *E. cancerogenus*, *Salmonella enteritidis*, *S. bongori*, *S. choleraesuis*, *Escherichia coli*, and *Serratia rubidae* [68]. Furthermore, *Bacillus cereus*, *Clavibacter michiganensis*, *Curtobacterium luteum*, *Microbacterium aerobescens*, *Microbacterium imperial*, and *Ochrobactrum antropi* were the pre-dominant bacteria in cassava leaves, followed by gram-negative bacteria such as *Pseudomonas rhodesiae* and *Enterobacter cloacae* [68], as shown in **Table 1**. They also demonstrated that environmental factors largely determined the phyllosphere’s microbial composition.

Interestingly, *Bacillus pumilus* isolated from stem cassava was considered as a biocontrol agent with anti-fungal activity in a detailed study conducted by Melo, Fiore [68]. This rod bacteria produces pumilacidins A–E, as shown in **Figure 2**. The molecular formulas of pumilacidin A, B, and C are $C_{54}H_{95}N_7O_{13}$, $C_{53}H_{93}N_7O_{13}$, and $C_{56}H_{99}N_7O_{13}$, respectively. Moreover, pumilacidin D and E share a molecular formula of $C_{55}H_{97}N_7O_{13}$. However, the amino acid valine was substituted for ileusin in pumilacidin D, resulting in pumilacidin E.

Another study from Canova, Petta [70] discovered that *Paenibacillus* sp. IIRAC-30 from cassava could produce a major surfactin C (**Figure 2**) compound with the molecular formula $C_{53}H_{93}N_7O_{13}$ and a $[M + H]^+$ on peak at m/z 1037.0. This strain also produces surfactin A ($C_{51}H_{89}N_7O_{13}$, the $[M + H]^+$ on peak is at m/z 1036.9) and surfactin B ($C_{52}H_{91}N_7O_{13}$, the $[M + H]^+$ on peak is at m/z 1022.9) as shown in **Figure 2**. These three secondary metabolites showed antifungal activity.

In addition, using a similar approach, Leite, Pereira [71] discovered 24 bacterial endophytes in cassava stems. According to Leite, Pereira [71] the most common genera discovered in this study were *Achromobacter*, *Bacillus*, *Burkholderia*, *Enterobacter*, *Pantoea*, and *Pseudomonas*. The majority of them demonstrated a variety of biological activities related to cassava plant growth and productivity [71]. In studies conducted by Teixeira and Vieira [72] and Teixeira, Melo [73], several

Genera	Stems	Leaves
<i>Bullera</i>	V	V
<i>Fusarium</i>	V	V
<i>Alternaria</i>	V	V
<i>Cryptococcus</i>	V	V
<i>Saitoella</i>	V	V
<i>Pseudopezyma</i>	V	—
<i>Ramichloridium</i>	V	—
<i>Aeurobasidium</i>	V	—
<i>Colletotrichum</i>	V	—
<i>Hannaella</i>	V	—
<i>Phaeosphaeriopsis</i>	—	V
<i>Pseudocercospora</i>	—	V
<i>Nigrospora</i>	—	V
<i>Aureobasidium</i>	—	V
<i>Pyrenochaetopsis</i>	—	V
<i>Sphaerulina</i>	—	V

Table 1.
Bacterial genera in cassava stems and leaves [69].

Figure 2.
Natural products produce by endophytic bacteria in cassava.

endophytic bacteria from cassava were identified, including *Bacillus*, *Burkholderia*, *Enterobacter*, *Escherichia*, *Salmonella*, *Serratia*, and *Stenotropomonas*.

Using a cultivation-dependent approach, Hartanti, Susanti [74] successfully cultured 14 endophyte fungi from cassava plants. All of them were examined using the ITS rDNA primers ITS 5 (forward: 5'-TCCTCCGCTTATTGATATGC-3') and ITS 4 (reverse: 5'-TCCGTAGGTGAACCTGCGC-3'). *Aspergillus* sp., *Aspergillus fumigatus*, *Fusarium*

Genera	Stems	Leaves
<i>Enterobacter</i>	V	V
<i>Pantoea</i>	V	V
<i>Pseudomonas</i>	V	V
<i>Escherichia</i>	V	V
<i>Stenotrophomonas</i>	V	—
<i>Aeromonas</i>	V	V
<i>Chloroplast</i>	V	V
<i>Klebsiella</i>	V	V
<i>Paenibacillus</i>	V	—
<i>Shigella</i>	V	V
<i>Lelliottia</i>	V	—
<i>Acinetobacter</i>	V	V
<i>Exiguobacterium</i>	V	—
<i>Erwinia</i>	V	V
<i>Methylobacterium</i>	—	V

Table 2.
Fungal genera in cassava stems and leaves [69].

falciforme, *Fusarium lichenicola*, *Fusarium oxysporum*, *Fusarium solani*, *Lasiodiplodia* sp., *Nectria pseudotrichia*, *Penicillium citrinum*, and *Schizophyllum commune* were discovered in this study [74]. Using similar approach, Suciati and Supriyati [75] successfully discovered *Guignardia endophyllicola*, an endophytic fungus, in cassava stems.

Zhang, Zhang [76] used a cultivation-independent approach of shotgun metagenome sequencing to determine the microbiome composition of cassava stems and leaves. The cassava phyllosphere's key bacterial genera have been identified as a result of this research. Gram-negative bacteria *Lelliottia* and *Stenotrophomonas* were isolated from cassava stems, gram-positive bacteria *Exiguobacterium* were isolated from leaves, and gram-negative bacteria [76], as shown in **Table 1**, the most prevalent genera were *Methylobacterium* from leaves. Therefore, the major fungi genera appear to be more complex than previously believed. Zhang, Zhang [69] discovered *Pseudopeziza*, *Ramichloridium*, *Aureobasidium*, *Colletotrichum*, and *Hannaella* were among the key fungal genera identified from cassava stems as shown in **Table 2**. Six fungal genera were discovered in the cassava leaves, including *Phaeosphaeriopsis*, *Pseudocercospora*, *Nigrospora*, *Aureobasidium*, *Pyrenochaetopsis*, and *Sphaerulina* (**Table 2**). In-depth analysis showed that *Bullera*, *Alternaria*, *Fusarium*, *Cryptococcus*, and *Saitolla* were identified in both phyllosphere samples [69].

In general, bacteria, fungi, and other microbes migrate into the plant phyllosphere via rain water, air, seeds, pollution, and animal sources [77]. Additionally, research indicates that some of these microbes are passed down from generation to generation [78]. The distribution of microbiomes in the phyllosphere may vary due to nutritional heterogeneity, such as carbon source uptake [79].

4. The rhizosphere and its microbiome

The soil ecosystem is one of the most complex and diverse on the planet. The soil contains a complex microcosm that interacts with the roots of plants [80].

This category includes archaea, bacteria, filamentous fungi, yeast, bryophytes, lichens, and protozoa. This soil organism significantly aids in the growth of various plants. Complex biochemical processes, such as the release of essential substances from organic matter, enable plants to access nutrients such as nitrogen, sulfur, and phosphorus, as well as essential growth hormones and toxic degradation products [81]. Furthermore, by providing pathogen protection, non-pathogenic microbes can alter plant immune responses [82].

In general, when plants live in a composite environment, they interact with specific soil microorganisms that live in the rhizosphere, the region around their roots [83]. This compartment is the narrow area of soil immediately surrounding the root system where the plant and microbes interact. It is defined by biological, chemical, and physical gradients that vary radially and longitudinally along the roots. The plant microbiome beneath the ground may be constructed in two stages: first, the rhizosphere may be colonized by a subset of bulk microbial communities, and then the rhizoplane (root surface) and root endosphere may be colonized by a subset of the rhizosphere communities [84].

Thousands of distinct microbial communities, including pathogens, mutualists, and commensals, coexist in the rhizosphere of cassava roots, just as they do in other plants. Their connection to the rhizosphere is complex and dynamic. However, it may be facilitated by the root exudate produced by the plant. Exudates play a critical role in plant–soil feedback by regulating plant survival in the face of antibiotic and biotic stress [85]. To the detriment of neighboring plants, plants regulate the rhizosphere via root-secreted metabolites [86]. Additionally, it is a critical mechanism of communication between plants and soil microbes [87]. The majority of root exudation takes place at the root's tip [88]. The root tip is the first part of the plant to investigate a new soil environment, and it plays a critical role in root responses to environmental stimuli [88]. Roots secrete a diverse array of primary metabolites, including amino acids, growth factors, vitamins, fatty acids, hormones, and antimicrobial compounds, which are believed to be lost passively from the root and utilized by rhizosphere-dwelling microbes [89].

Additionally, via a complex mechanism, exudates play a critical role in shaping microbial diversity [90]. However, no specific research on the microbial shaping of cassava plants in response to exudate has been conducted. However, research on other plants may explain this discrepancy.

Bacillus, a genus bacteria, in tomatoes produce systemic exudates of acylsugar metabolites, as demonstrated in a study of Korenblum, Dong [91]. Additionally, the metabolomes and transcriptomes of tomato leaves and systemic roots change in response to the rhizosphere's microbial community structure [91]. In-depth analyses of the systemic root metabolome suggest that glycosylated azelaic acid may function as a signaling molecule that is induced by the microbiome and then excreted as free azelaic acid [91]. The results indicate that the rhizosphere microbiome assembly plays a molecular and chemical role in systemically induced root metabolite exudation and soil conditioning.

Another study by Strehmel, Böttcher [92] reported that when *Arabidopsis thaliana* was grown hydroponically, it produced over a hundred distinct metabolites belonging to a variety of chemical classes. This metabolic diversity suggests that plants have developed a sophisticated chemical language for mediating an infinite number of rhizosphere interactions [93]. In conclusion, these studies indicated that structural changes in microbial communities have the potential to significantly alter host phenotypes. Additionally, root exudates have the potential to act as messengers between roots and soil organisms, triggering biological and physical interactions.

Melo, Fiore [68] used a cultivation-dependent approach to successfully cultivate 27 endophyte bacteria from cassava root. According to this study, *Bacillus*

predominates in cassava root [68]. Gram-negative bacteria (*Kluyvera cryocrescens*, *Stenotrophomonas maltophilia*, *Enterobacter aerogenes*, *Klebsiella pneumoniae*, and *Acidovorax avenae*), gram-positive non-Actinobacteria (*Bacillus cereus*, *Burkholderia cepacia*, *Bradyrhizobium japonicum*, and *Microbacterium homonis*) and gram-positive Actinobacteria (*Streptomyces olivaceus*) were found in cassava root [68]. Another study by Leite, Pereira [71] used a similar approach to discover 28 bacterial endophytes in the root. The most prevalent bacteria found in cassava root were *Bacillus*, *Burkholderia*, *Enterobacter*, and *Pantoea*. The majority of them possessed biological properties, including the ability to solubilize inorganic phosphate and the capacity to synthesize Indole acetic acid [71].

Zhang, Zhang [76] successfully identified a variety of bacterial genera in the cassava root. Gram-negative *Enterobacter*, *Pantoea*, *Pseudomonas*, *Escherichia*, *Aeromonas*, *Chloroplast*, *Shigella*, and *Klebsiella* were discovered in cassava roots, as were gram-positive *Lactococcus* and *Paenibacillus* [69]. Therefore, the only major bacterial genera found in root cassava were gram-positive cocci *Lactococcus*. Additionally, using a cultivation-dependent technique, Ilyas [94] isolated endophytic fungi *Fusarium* sp. and *Penicillium* sp. from cassava roots.

A recent study took a non-cultivation-dependent approach. Zhang, Zhang [76] discovered 11 fungal genera in the cassava root, including *Bullera*, *Fusarium*, and *Alternaria*, as well as eight key fungal genera that were not found in the cassava stems and leaves, including *Humicola*, *Penicillium*, *Nigrospora*, *Beauveria*, *Thozetella*, *Codinaeopsis*, *Paraphaeosphaeria*, and *Dinemasporium* [76]. Additionally, *Ascomycota* have been described as domination endophyte assemblages. According to a study conducted by Li, Yan [95], *Stephanonectaria*, *Cutaneotrichosporon*, *Pleurotus*, *Wallemia*, *Aspergillus*, *Gibberella*, *Lachancea*, *Yamadazyma*, *Neurospora*, *Cladosporium*, *Wickerhamomyces*, *Penicillium*, *Diaporthe*, *Fusarium*, and *Lasiodiplodia* were successfully detected in cassava root. Therefore, *Lasiodiplodia* was genus-level dominant [95].

5. The effect of plant genotypes and genetic background on plant microbiome

Plants live harmoniously with a diverse array of microorganisms. These microbes, which include bacteria, archaea, filamentous fungi, and nematodes, can live as endophytes or epiphytes, as well as in any plant organ or tissue, including cassava. A rapidly growing body of literature has documented the influence of the microbiome on critical plant traits such as disease resistance [96], nutrient acquisition and growth [97], and abiotic stress tolerance [98]. Thus, the microbiome can be viewed as an extended phenotype of the plant genome that can assist plants in dealing with environmental stressors.

Li, Yan [95] investigated the microbiome of various cassava cultivars in a study. They examined four cassava cultivars, two of which were resistant to rot (SC124 and SC205) and two of which were susceptible to rot (SC124 and SC205) (SC10 and SC5). Surprisingly, both groups were dominated by gram-positive *Weissella* (family *Leuconostaceae*) close behind with gram-negative *Serratia* (family *Enterobacteriaceae*). At the phylum level, the most prevalent phyla were *Proteobacteria* and *Firmicutes* [95]. Thus, *Lasiodiplodia* (family *Botryosphaeraceae*) was the most prevalent fungus in the susceptible and tolerant groups, followed by *Fusarium* from family *Nectriaceae* and *Diaporthe* from family *Diaporthaceae* [95]. Thus, susceptible cultivars have been found to harbor bacteria such as *Paenicaligenes*, *Parapusillomonas*, *Corticococcus*, and *Lachinoclostridium* that have not been detected in tolerant cultivars [95]. On the other hand,

Phascolarctobacterium, *Olivibacter*, and *Citrobacter* were key genera found exclusively in the tolerant group [95]. *Culvularia* was the most frequently encountered fungus among vulnerable groups. *Hortaea* and *Agaricostilbomyces* were significantly more abundant in the tolerant cultivar, indicating the importance of relative abundance [95].

Zhang, Zhang [69] is also investigating the microbiome of cassava plants that is associated with disease resistance. Interestingly, several microorganisms involved in disease resistance include *Lactococcus* sp., *Pantoea dispersa*, and *Saccharomyces cerevisiae* [69]. Additionally, the presence of nisin-related genes in *Lactococcus* was positively associated with disease resistance in cassava plants [69].

6. Manipulation of cassava microbiome to improve the yield

Like in other plant, manipulation of the plant microbiome may aid in increasing cassava productivity [99]. By increasing soil bioavailability and plant tolerance to biotic and abiotic stresses, good soil management practices such as the use of beneficial microbes in the Rhizosphere can be achieved, thereby reducing reliance on agricultural chemicals. Crop rotation is also an option for increasing the diversity of soil microbes, which contributes to plant pathogen resistance [100]. A stimulating biofertilizer as shown in **Figure 3**, which includes co-inoculation of several beneficial strains, including endophytes, will enhance microbial root colonization capability and establish a useful niche for plant pathogens to compete. *Bacillus pumilus* and *Paenibacillus* spp. inoculation will improve fungal pathogen suppression on cassava plants as a biofertilizer agent capable of producing pumilacidines and surfactins. Additionally, inoculants containing microorganisms and microbial phosphorus solubilizers capable of producing active indole acetic acid promote the growth of manicured plants (as shown in **Figure 3**).

Figure 3.
Consortium's inoculant strategy for improving the cassava microbiome and production.

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