



Microbiome Engineering for Sustainable Agriculture

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ABSTRACT

Microbiome engineering represents a promising frontier in sustainable agriculture, leveraging plant-associated microorganisms to enhance crop growth, nutrient uptake, and resilience to environmental stressors. This paper explores the definition, significance, and diverse applications of microbiomes in agricultural systems, emphasizing their role in improving soil health, promoting plant growth, and reducing dependency on chemical inputs. Techniques such as metagenomics and metatranscriptomics are discussed as tools for understanding and manipulating microbial communities to achieve sustainable outcomes. The potential of microbiome engineering to address global agricultural challenges is immense, yet it faces obstacles related to microbial community establishment and regulation. Future directions in research and field applications are highlighted to harness microbiome engineering's full potential for agricultural sustainability.

Keywords: Microbiome engineering, sustainable agriculture, plant microbiomes, metagenomics, metatranscriptomics.

INTRODUCTION

Microbiome engineering is increasingly being leveraged for sustainable agriculture. Microbes are living bacteria, fungi, viruses, and other microorganisms that symbiotically reside in and on plants. They have coevolved over millions of years with their host plants into complex and stable communities, influencing nutrient uptake, boosting the immune responses of plants, and ensuring exposures to other harmful microorganisms and foreseen changes. Commercial interests and academic research have largely developed around microbiome engineering in humans, ignoring its significance in plants. Over the years, an increasing number of field studies have provided conclusive evidence that plant microbiomes have an overwhelming influence on crop growth and farmlands' health. Microbiome's ability to engineer the growth and physiology of plants through biofertilization and bioprotection, adaptability, speed, and variable outcomes makes it an exciting non-genetic option for agriculture sustainability [1]. Novel agriculture systems are an essential farming strategy in the face of an increasing population and shifting climates. These systems could revolutionize the worldwide agricultural industry by achieving high output, food security, and ecological sustainability. Microorganisms could provide crops with the ecosystem benefits required to manage these novel agriculture systems effectively. Simultaneously, problems associated with the complex reestablishment of native or genetically tailored microbiomes in the farming locations of vastly varied topographical scales need to be solved. The application of microbiome engineering is envisaged in various research articles to address these challenges for the sustainable agriculture of the 21st century [2].

DEFINITION AND IMPORTANCE OF MICROBIOME IN AGRICULTURE

A microbiome is the entirety of microorganisms, the surrounding environment, and the interactions between them within a given niche. In agricultural systems, microbiomes are particularly important due to their dynamic interactions with crops, available resources, and the environment. They can affect the health and resilience of the system either negatively or positively. A healthy microbiome increases the ability of the crop to cope with environmental stress and limits the need for external input, such as harmful pesticides. It plays a crucial role in plant growth promotion and resilience to nutrient deprivation, making it an important target for the transition towards sustainable agriculture.

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Understanding and utilizing microbiomes in agriculture corresponds with the increasing global concern for food security, climate change, and the environmental impact of agricultural processes, as microbiomes can contribute to the overall productivity, nutritional quality, and sustainability of crop production [3]. The collective genomes of the microbiota in any given biological niche are referred to as the metagenome. The collective gene products - the metagenome - can be referred to as the functional gene content, and is an important consequence of collective metabolic potential. In agriculture, many studies focus on the rhizosphere microbiome due to its important role in nutrient acquisition and plant productivity, and it is a highly dynamic environment due to the supply of carbon-rich root exudates. In other systems, microbiomes play a role in several important functions related to crop physiology and the soil ecosystem, and include the endosphere and phyllosphere [4].

MICROBIAL DIVERSITY IN AGRICULTURAL SYSTEMS

The diversity of microorganisms, comprising bacteria, fungi, nematodes and their parasites as well as husbandry animals, can provide a range of benefits to agricultural systems. A range of wastes can be a potential source of environmental contaminants, including pathogens. Some research has been conducted into the movement of pathogens with wastes from intensive cage layer farms. Few have predicted the spreading of contamination through airborne movements [5]. Vast microbial diversity in soil ecosystems is a key contributor to soil health and enables the myriad of ecosystem services provided by the soil. The focus of soil health is now of interest to the development of sustainable agricultural practice as intensified, monoculture-based agriculture is causing great ecological distress. As eighty percent of the biomass of soil microorganisms is unidentified, it is not possible to fully understand how any given microorganism continues to maintain ecological balance or prevent more precise over-intensive, sometimes military, parochialistic 'agriculture' ('agro' = fields and the French word to cultivate is 'culturer'). However, to isolate those organisms, the specific soil and management conditions apply to their activity. It is often illegal to keep this data secret. It is only through the engagement of the greater scientific community that the true implications of such organisms, not just for traditional high yield monoculture, could begin to be appreciated [6].

Key Microbial Players in Soil Health

Compatibility, soil structure, soil moisture, and in turn plant productivity and farmer economic returns. Realizing the pivotal roles that soil microbiomes can play in some of the most intractable problems of agriculture, the Agriculture 4.0 paradigm in the 21st century is envisioned to leverage such microbiome players toward delivering sustainable agriculture. Already, scientific advances, fueled by the availability and dissemination of knowledge generated by large microbiome data sets, has already catalyzed the development of microbiome-based products to improve soil health or enhance plant nutrient uptake, and thus manage root disease in the field. The majority of such efforts have focused on 'bottom-up' approaches, where environmentally-selected bacterial isolates are used to expand the microbiome of plants and soils to promote beneficial outcomes [7]. In this paper, we focus on 'top-down' methods that start from the soil as source. The complex soil microbiome can express function that make a difference within the larger agro-ecosystem; the goal of microbiome engineering encompasses both deliberate engineering and culturing of bacteria that show broadscale evidence of increased performance in many agricultural contexts, and deliberate development of plant varieties whose root tissues invite and promote those microbial functions into their root ecosystems. Shallower parts of this paper delve into the key ecological principles needed to link functions, bacteria, and benefits to their hosts in order to tilt the ecosystem more deliberately towards positive experiences for farmers [8].

TECHNIQUES FOR ENGINEERING MICROBIOMES IN AGRICULTURE

Global-scale challenges in agriculture, such as maintaining crop yield in the face of a changing climate while reducing the inputs of chemical fertilizers to mitigate environmental impacts, pose complex and interlinked problems to researchers across many related fields. Decades of research have shown that the communities of plant-associated microorganisms affect host performance by mobilizing and cycling carbon and nutrients. Recently, the introduction of new microbiomes by synthetic microbial inoculants was proposed to manipulate soil microbiota and improve crop yield. Technologies applied in microbiome engineering that are based on advanced techniques in microbiology and genetics have wide-reaching applications across agriculture, one of the most complex and applied cross-sections of microbiome research [9]. There are many methods to engineer plant microbiomes that can be split into two broad categories: the first where a microbial community is created in vitro and then applied to a host, and the second where the host is treated with different growing conditions and the indigenous microbial community is manipulated and then applied to (or replaced with) another host. Metagenomics (sequences DNA) and metatranscriptomics (sequences mRNA) are the sequencing of genetic material from all

members of a complex microbial community and subsequently provide insights into the functional potential of the microbes. Alternatively, initial denaturing gradient gel electrophoresis (DGGE) and then 16S rRNA gene sequencing were used to profile the microbial community and inform the design of synthetic communities. The multiple sequence tags were annotated, with the predicted gene functions providing an idea of the community high-level functions. Therefore, as sequencing technology has advanced, so has the resolution of the tools used in microbiome and soil microbiome engineering [10].

Metagenomics and Metatranscriptomics

Metagenomics is a system-level approach that discards the need to first associate function to specific microbes. Because metagenomics sequences the nucleic acids from all the microbial community members in an environment, and researchers decode the functional potential of the community based on these sequences collected. It's a new, whole-ecosystem approach to understanding community interactions based on their gene functions. This approach can pave the way for dietary recommendations to apply at the level of the whole ecosystem, that aim to positively where the genetic potential of the microorganisms and provide microbial taxa that would be well adapted to the habitat and maximize performance from feed metabolism [11].

Metatranscriptomics, a direct extension of metagenomics, couples function to gene activity by sorting genes based on gene expression. A transcriptionally active gene has a direct impact over the metabolic pathway of the organism which synthesizes it and RNA sequencing can provide qualitative data on the linkage of genes and enzymatic activity which is involved in nutrient and energy flow through metabolic pathways and thus provides more complete functional capacities of the ecosystem and more diagnostic information that genetic data. Hernández-Sanabria et al., 2012 were able to measure the expression of genes in bacteroidetes, se bacteria, proteobacteria and clostridia in the bovine rumen using metatranscriptomics [12]. This ecosystem-based approach allows us to engineer communities at the microbial level within the microscope network instead of relying on single species interventions, metagenomic and/or metatranscriptomic technologies would in theory be able, at least for the neighbor functions in the current and natural microbiome, to efficiently guide these long-term process of play selection and could help select, engineer and even replace with another strategy. The microbiome research has made it possible to greatly understand an organism's genetic potential, but we still have a lot to learn about his "gene machinery" and systematic ways on how to improve it [13].

APPLICATIONS OF MICROBIOME ENGINEERING IN SUSTAINABLE AGRICULTURE

Applications of microbiome engineering in sustainable agriculture Since fundamental scientific evidence assures that modulation of bacterial communities in the plant microbiome plays a central role for successful plant growth in sustainable agriculture, microbiome engineering is attracting more attention for its practical applications. For example, changes to the root-associated microbiota reportedly enhance nitrogen, phosphorus, and other nutrient uptake, promote pest resistance, and improve drought tolerance and plant growth. This section covers diverse microbiome engineering applications with the main objective to enhance nutrient uptake and promote plant growth. The positive effects on uptake, transport, and accumulation of essential nutrients will, of course, likely increase the plant yield [7]. Based on the intertwined mechanisms of bacterial communities with nutrient uptake, this section gives an overview of such a microbiome engineering benefit in the crop microbiome. Bacterial inoculants that promote phenotypic changes now include long-life bacteria such as *Mycobacterium* and companies such as CollectiveCrunch, which specialize in organizing the bacterial community and interpreting genetic and phenotypic changes. The overall outcomes of microbiome engineering benefit would include productivity and environmental sustainability. The potential for microbiome engineering to create a sustainable environment rekindles some hope that the dire consequences of overuse of chemical fertilizers and pesticides can be limited. Microbiome engineering helps eliminate agricultural input pollution, strengthens agriculture as growing population pressure triggers land degradation, and supports food safety and security. Complement or supplementation of soil-improving or plant empowerment bioproducts may greatly contribute to increasing the uptake, leading to significant increases in agricultural productivity and a decrease in the ecological risk of excessive input use. In a word, microbiome engineering is anticipated to be cost-effective in solving agricultural sustainability issues [14].

ENHANCING NUTRIENT UPTAKE AND PLANT GROWTH

At the root level, microorganisms colonizing plants can have diverse roles and functions. Microbiome engineering through inoculation of beneficial microbes and microbe-derived products is a promising strategy to improve nutrient uptake, facilitate plant growth, and enhance phytoremediation. A system dedicated to taking up nutrients can consist of monocultures or polycultures, can be supported or not, and

can be irrigated or not, to give a few examples. All resources are considered without any limit; for example, water can be used to leach the salts, whereas the excess nutrients can be dealt with or are an intrinsic part of the agriculture system. Engineering the rhizosphere to improve plant growth and nutrient uptake via microbe-based formulation or probiotics emerges as a prioritized goal for improving agricultural sustainability. In more recent work, agricultural cultivation with the primary focus of nutrient extraction, specifically for N, Fe, or both, has been proposed as an integrative strategy for in situ biomanufacturing of nanoparticles through beneficial bacteria in engineered rhizobacterial consortia. Such N-deficient aquaponic systems can serve as nutrient-linked food-energy-water nexus lifelines where agriculture is geared toward the cultivation of specialist plant communities capable of driving closed-loop systems that facilitate selective color-matched residential or community-based N-rich waste inputs to maintain or augment loop closure [7]. Auxins and gibberellins are classes of plant-growth-promoting hormones that are synthesized mainly in the root phyllosphere and act as signaling molecules that may affect the plant microbial community. Microbial auxins are similar to the plant hormone indole acetic acid, which regulates plant growth and development, induces adventitious roots, and decreases the consequences of transplant shock. Gibberellins impact virtually every part of the plant, including stem and root length, flowering, fruit set and development, and seed germination. Nitrogen fertilization has a significant impact on the composition and abundance of aboveground and belowground microflora. Fertilized soils stimulated saprophytic bacteria, such as Actinomycetes, that excrete antibiotics. Similarly, an increased quantity of antifungal clay was observed in fertilized fields. Microbiome engineering aims to boost plant immune responses by introducing biocontrol agents, inhibiting disease-causing pathogens, and therefore overcoming some of the harmful effects of fertilization [15]. Plant diseases continue to cause serious crop losses. Regular pesticide use to prevent pathogenic microbes that cause various diseases is detrimental to the ecosystem and the environment. Biological control has advantages, and plant growth-promoting microorganisms supply crops with nutrients, help plants deal with stress, and decompose matter. In recent years, nonpathogenic microbes have been used as potential control agents for plant diseases based on microbiome-based mechanical engineering. These include the use of living microorganisms such as bacteria and fungi and microbial derivatives such as bioactive compounds, cell-wall-degrading enzymes, and volatile organic compounds [16].

CHALLENGES AND FUTURE DIRECTIONS IN MICROBIOME ENGINEERING

Molecular technologies can be used to engineer microbiomes to increase crop tolerance and productivity. In this review, we introduce some general advantages and challenges of microbiome engineering for sustainable agriculture and in-field responses of field trials. Microbiome engineering can be applied to provide stable crop responses even in frequent stressful conditions. It is, however, uncertain whether genetically modified (GM) bacteria can colonize the root zone in competition with native bacteria over a long period of time. PM185, a Gram-negative bacterium that over-produces the osmoregulator trehalose, provided stable produce benefits in field trials at four different American vegetable farms in response to two different kinds of abiotic stress [17]. Microbes that are modified by recombinant DNA technologies to better perform as bio-fertilizers or as bio-pesticides are usually called bio-synthetically engineered microbes (BSEMs). The key innovations in BSEM research are the outcome of years of microbial ecological research demonstrating the many benefits of harnessing microbiomes (also called microflora) for societal goals. Microorganisms in soil microbiomes can aid plant growth. This can occur through production of plant hormone stimulants, which can include gibberellins or more biologically and chemically complex molecules like pyocyanin produced by fluorescent *P. Fig* widespread, antifungal phenazines produced by *Pseudomonas chlororaphis*. When pathogens attack, beneficial drought and salt tolerant microbiomes made up of many microbes can produce metabolites taken up by the roots, such as the osmoregulator trehalose, an unusual sugar-like molecule that lowers the free water activity of microbe cell, and bacteria and root tissue up to 1:10 trehalose: water. This dramatically lowers the humidity of water inside the cells of the pathogen microbe assailing root and leaf surfaces, so water floods out of pathogen cells which begin to rapidly desiccate and die before they have time to multiply in the affected part of the plant [18-19].

CONCLUSION

Microbiome engineering offers transformative potential for sustainable agriculture by optimizing the interactions between plants and their associated microorganisms. This approach can enhance nutrient uptake, promote plant growth, and provide resilience against pests and environmental stressors, thereby reducing the need for chemical inputs and mitigating their negative impacts. Advances in metagenomic and metatranscriptomic technologies have facilitated deeper insights into microbial communities, enabling targeted manipulation for improved agricultural outcomes. While challenges such as microbial

community establishment and regulatory considerations persist, continued research and innovation hold promise for overcoming these barriers. Ultimately, microbiome engineering stands as a crucial strategy in the quest for agricultural sustainability, contributing to food security and environmental stewardship.

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