

Review Article

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MAP's Assisted Microbiome Engineering

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ABSTRACT

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Green revolution is considered as greatest landmark in the history of agriculture, as it has saved nearly 25 million hectare of land being converted to agriculture and prevented billions of peoples from starvation. However, over exploitation of agrochemicals like pesticides and chemical fertilizers have resulted in to sever health issues for example majority of peoples in Punjab region are suffering from cancer and other health problems. Also it has resulted in to pollution of water bodies and has deteriorated quality of land and have degraded the soil health, thereby bringing unsustainability in long term. So, what can be the solution which can bring sustainability in medium to long term without affecting the natural ecosystem? Solution is Plant Microbiome, engineering plant microbiome by using diversity to grow more.

Introduction

Green revolution is considered as greatest landmark in the history of agriculture, as it has saved nearly 25 million hectare of land being converted to agriculture and prevented billions of peoples from starvation. However, over exploitation of agrochemicals like pesticides and chemical fertilizers have resulted in to sever health issues for example majority of peoples in Punjab region are suffering from cancer and other health problems. Also it has resulted in to pollution of water bodies and has deteriorated quality of

land and has degraded the soil health, thereby bringing unsustainability in long term. So, what can be the solution which can bring sustainability in medium to long term without affecting the natural ecosystem? Solution is Plant Microbiome, engineering plant microbiome by using diversity to grow more.

Microbiome is a community of microorganisms (such as bacteria, fungi, and viruses) that inhabit a particular environment and especially the collection of microorganisms living in or on the plant, human or any living organism's body. Plant

Microbiome comprises of the microbial community which inhabits different plant organs like, root (Rhizobiome), leaf, stem, flower etc.

Holobiont: Organism together with its microbiome comprises holobiont.

Phytobiont: Plant along with its complete microbiome is regarded as Phytobiome.

How plant recruits its microbiome naturally

Plant follows the following strategies:

Production of specific root exudates e.g. Malic acid, succinic acid.

Secretion of secondary metabolites e.g. DIMBOA by maize plant.

Exudation of signaling molecules e.g. AHL by *Medicago truncatula*.

Plant genotype e.g. Glucosinolates production by transgenic Arabidopsis.

Defense activation and recruitment in response to infection e.g. Firmicutes against *Ralstonia solanacearum*.

How we are shaping the microbiome till date

Selection during domestication of crop plants.
Plant breeding to change quality and quantity of root exudates.

Development of transgenic varieties.

Bio-fertilizers: introduction on seed, planting material or in soil.

Foliar spray of nutrient, organic compound and chemicals.

Crop management practices.

Soil type and properties

MAP's in engineering plant microbiome

MAP's stands for microbiome assisted engineering. It is qualitative and taxonomy driven approach. These emerging MAP's includes the traits like Plant growth, root architecture, flowering time, drought

resilience and disease suppressiveness. This taxonomic and functional basis of MAP's can be elucidated through amplicon sequencing, isolation and phenotypic screening and by using the shotgun omics approaches like Metagenomics, metatranscriptomics, Metaproteomics combined with the studies of metabolomics which will give a complete overview of metaphenome of a plant.

Map's assisted plant microbiome engineering involves two approaches. First top down approach involves mathematical and experimental analysis for quantitative assessment of potential and ecological context of MAP's. Unlike top down approach, bottoms up approach involves targeted analysis of host and microbes for identification of functional basis of MAP's at molecular and biochemical level.

MAP's first approach

The main guiding principle in MAP's first approach is going back to the roots which involves search for the missing plant microbes to restore the plant microbe interactions lost during domestication or during breeding for disease resistance. This MAP's first approach involves systematic quantification of most significant MAP's across and wild and domesticated host, herein natural ecosystem, traditional and modern agriculture serves as reservoir of genetic and ecological potential, for identification of microbiome associated phenotypes. These MAP's are systematically screened for identification of plant microbe and environmental combinations in which MAP's provide largest fitness advantage. This framework will guide into the mechanism that drives MAP's, and this generated information can be used for targeted plant breeding and microbiome engineering in concert with the plant genotype referred to as Next generation agriculture.

Experimental basis of MAP's

MAP's are defined quantitatively, for examples salt tolerance, phosphorus solubilization, disease suppressiveness etc. but the contribution of MAP's to fitness and the conditions in which maximum benefits from cumulative microbiome effect comes should be assessed quantitatively. These studies will inform us about where to target the mechanistic investigation, where to apply direct microbiome engineering efforts and it will also guide the field applications for results generated and applications developed. Once a particular MAP has been determined, it becomes necessary to assess the cumulative microbiome effect across range of conditions to determine the effect on host in both germ free and microbiome associated host states.

For each conditions, fitness can be assessed quantitatively using parameters such as yield, number of lateral roots, chlorophyll content etc. This experimental basis or setup can be used for studying the changes in the fitness of host plant having similar genotype but differing in their microbiome composition by subtracting or removing the fitness of host in germfree condition from the fitness of microbiome associated host, which will provide insights into the maximum cumulative microbiome effect.

A condition of Disbiosis can be observed in the case of plants under differing salt concentration, where the fitness of germfree host plant is more compared to the fitness of microbiome associated host plant. This condition of Disbiosis is the result of disbalance in microbiome composition or functioning causing negative effect on host fitness and phenotypes. Also this experiments can be exploited for studying the effect of two different microbiomes on the same plant keeping all other parameters same.

For successful adaptation of microbiome, taxonomic shift and functional changes are of critical concern. This host guided selection experiments can provide insights into the additional parameters such as, holobiont recruitment rate and holobiont fitness stability. Thus this experimental basis shows that optimizing variables such as, altered cumulative microbiome effect, holobiont recruitment rate and holobiont fitness stability, to achieve maximum fitness gain and rapid recruitment and stability are key objectives of engineering microbiome. Based on these findings targeted comparative analysis selects for most significant emergent MAP's. From microbial side analysis of functional and compositional enrichment and significant co-occurrence pattern can be used for design of synthetic microbial community.

The most significant microbial and plant traits combined with the Genome wide association studies, Exudate profiling, screening and preparation of isolate libraries along with holobiont omics and QTL analysis, will provide genetic, molecular, ecological and biochemical signatures for holobiont engineering.

Modular microbiome

What is module?

Discrete, individually separate and distinct, functional communities may be viewed as modules. Modules can be combined to provide novel functional combinations, which are designed to increase the host fitness across the multiple niche dimensions. Oyserman in 2016 provided the functional basis of modularity in microbiome when he tried to develop a novel waste water treatment by combining photosynthetic nitrifying group with polyphosphate accumulating organisms group. The concept was to use the full potential of each functional guild's unique

metabolic potential. By designing such biogeochemically complementary communities or modules, the need for mechanical aeration was overcome and polyphosphate cycling was not interrupted.

From this experiment it becomes clear that, the impact of different communities or modules on the function is nonlinear. Some communities can be dominant with no significant change in cumulative microbiome effect and some are recessive with significant change in cumulative microbiome effect after mixing due to trade of between the two functional MAP's, which constitutes a suboptimal space and this can be represented by Pareto curve, such that increase in $\Delta A1$ occurs with trade of or at the cost of decrease in $\Delta A2$. By designing the microbiome with multiple modules, the slope of the Pareto curve can be shifted such that, increase in $\Delta A1$ and $\Delta A2$ can be achieved with a cost to the other.

Successful implementation of microbial communities as modular component requires, firstly, minimizing the overlap in resource requirement between the functional guilds, example between endosphere and rhizosphere. Secondly minimizing the trade of between the functional guilds, which will be the key for developing customizable and modular microbiome.

Because there is no any silver bullet organism which will provide an optimal MAP under all environmental and ecological conditions, modularity in microbiome would provide agriculture with toolbox to rapidly adapt and maximize the crop yield under diverse environmental and ecological conditions.

How the modularity in microbiome can be achieved?

The impact of modular microbiome on fitness can be quantified using the host guided

selection to find optimal positions in Map's solution space by "changing the rules of the games" through, resource partitioning of the root exudates such that separate functional components of microbiome can coexist. e.g. Plant microbe cross feeding strategies in opine producing plants, which preferentially selects for opine catabolizing microbes and minimizes the cross talk with non-target microbes. Alternatives can be

1. Breeding for tailored root exudates composition and designing functional microbial modules depending on these distinct exudate profiles.
2. Exploiting natural compartmentalization produced by rhizosphere, endosphere and phyllosphere communities.
3. Engineering MAP heterogeneity at population level, can lead to intercompatibility between MAP's without necessitating individual host level modularity e. g. an approach analogous to intercropping can be adapted neighbors or interacting microbes provides combinatorial effect for the population.

In context to going back to the roots, microbiome modularity may already be a trait of many plants in the wild that faces changing conditions and natural selection, thus natural variation in host optimized genetically for millions of years by evolution may serve as yet untapped source for investigating microbiome modularity.

Network analysis of the community structure may be used to identify such modules including hub taxa that can form central node in the community network.

Functional basis of modularity

As we know the plant microbe interactions are mediated by molecules secreted by plant and microbes both, which may include,

primary metabolites e.g. Sugars and amino acids, and secondary metabolites like signaling and antimicrobial compounds. They may provide carbon source, complement auxotrophy, attract, deter or act antagonistically to dictate the microbiome assembly process. So to understand the functional basis we first need to understand the genetic basis and chemical interplay that drives the MAP's. This study will provide us

Specific markers for plant breeding.

Guide the design of microbial consortium.

Identify the prebiotic molecules incorporated into the granules or seed coating for inundative microbial application, which triggers the beneficial activities associated with particular MAP's.

But, the problem here is most of the root exudates are considered as public goods by all microbiota, which presents a high potential for cross talk. A good example of strigolactones can be taken in this regards. Strigolactones generally act as a signal to recruit plant beneficial microbes, but they are also hijacked by pathogens and parasitic weeds for germination, root attachment and root infection. Thus the development of mechanism to limit the signal poaching is important modular microbiome engineering.

The key challenges are to,

Understand the complexity of microbial traits that plant attempts to recruit.

Taxonomic distribution in microbiome.

Specificity of host signals needed to recruit the MAP.

What can be done to overcome these challenges?

Signal specificity may be achieved through precise combination of primary carbon sources provided in the exudates, auxotrophies and specialized metabolites that

can act as antimicrobials or as a signal for microbial chemotaxis.

Identification of such signals and connecting them to loci in plant genome which can be used in the plant breeding strategies to effectively select for microbiome associated traits.

Use of recombinant inbred lines to identify microbiome QTL, especially when provided with metabolic data, which can be harnessed to connect plant genotypes statistically not only to MAP's but also to plant metabolites that effectuate them through direct or indirect roles in host selection of microbiota.

In conclusion, there is a need for quantitative assessment of the relative contribution of the host genotype, microbiome and environmental conditions for a given phenotype. Mathematical MAP's first approach will provide a more solid basis for engineering microbiome to enhance plant growth and tolerance to a/biotic stresses. Identifying the vast and yet unknown functional potential in host associated microbiome and unraveling the dynamic chemical interplay between host and microbiome will be essential to elucidate to what extent and how host recruits or activates members in microbiome for their own benefit. QTL mapping for traits that support PGPR and understanding genetic basis of microbiome assembly can be explored to identify the traits that can be exploited for. Augmenting beneficial members of indigenous micro-flora in soil. To support and sustain modular microbiome that is introduced into a host system or population.

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