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Functional Manipulation of Root Endophyte Populations for Feedstock Improvement

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Introduction

Plant-derived carbon is the primary energy source for terrestrial heterotrophs, most of which are microbial. The interaction of these microbial heterotrophs with the plant ranges between the extremes of mutualistic symbiosis [1] and pathogenesis [2,3]. The vast majority of plant-associated microbial diversity, however, lies between these two extremes, inducing more subtle, context dependent effects on plant fitness [4–6]. This ecosystem, referred to as the plant microbiome, contributes to the extended phenotype of plants. It exerts a cost from the plant, by consuming photosynthate [7–9], and it provides benefits, via protection from pathogens [10–14] or abiotic stress [15,16] or by increasing nutrient bioavailability [4,17,18].

The prospect of developing generalizable methods of optimizing the plant microbiome for desired plant traits requires an understanding of the ecological rules and patterns that underlie its assembly. Access of a microbe to the root, which can be described both in terms of dispersal limitation and of source-sink dynamics, is likely correlated with its abundance in soil. Indeed, the community composition in soil has an immense influence on the composition of the plant microbiome [19–21].

A number of studies have attempted to correlate soil microbial community composition and diversity with soil abiotic factors such as pH [22–25], drought [25–28] and nutrient concentrations [24,25,29,30]. Data from these studies suggest that whereas pH is a key determinant of soil microbial biogeography, gradients in water availability and nutrient concentration produce significant shifts in microbial diversity as well. Single variables, however, cannot be completely isolated in natural surveys.

In order to bridge the gap between ecological observation and a mechanistic understanding of underlying processes, it is necessary to isolate niche processes contributing to community assembly in both soil and plant from the stochastic component of neutral processes. Niche sorting of plant associated communities is determined by three types of interactions: (a) abiotic interactions; (b) plant-microbe interactions and (c) microbe-microbe interactions. However, higher order interactions, such as the effect of abiotic conditions on plant-microbe interactions also need to be accounted for, as is evidenced in the interaction between plant phosphate starvation response genes and defense genes [4].

In order to control for these three types of interactions, we constructed plant-microbe microcosms, that can be deconstructed to their individual components, while retaining a complexity that is comparable to natural ecological communities. To model the Arabidopsis thaliana microbiome, we inoculated gnotobiotic plants with a synthetic community (SynCom) composed of 185 genome-sequenced isolates obtained from Arabidopsis roots. This model system allows measurement of abiotic interactions by manipulating chemical composition of the growth media and of biotic interactions by editing the SynCom. Using this system, we explored how four environmental gradients: temperature, salinity, pH and phosphate concentrations influence the assembly of bacteria into plant-associated communities. We used co-occurrence patterns of SynCom members across these gradients to guide experimental tests of microbe-microbe and microbe-plant interactions, and of the mechanisms by which these interactions contribute to bacterial community assembly and to plant phenotype.

Results

To measure the robustness of plant microbiomes to environmental variation and the influence of abiotic interactions in plant microbiome assembly, we exposed 7-day old A. thaliana seedlings, growing on agar plates with Johnson's media (JM), with or without a SynCom, to four environmental gradients: Salinity (50, 100, 150, 200 mM NaCl), pH (5.5, 7.0, 8.2), Pi concentration (0, 10, 30, 50, 100, 1000 µm Pi) and temperature (10, 21, 31˚C). After 12 days of growth under these conditions, bacterial DNA was extracted from four fractions: roots, shoots, agar, and unplanted agar. Bacterial community composition was determined by 16S rRNA amplicon sequencing, mapping reads to 97 unique sequences (USeq) representing the 185 strain-SynCom. In order understand the determinants of root and shoot colonization, we compared alpha- and beta-diversity between the plant tissues and agar and applied a generalized linear model (GLM) to ask which strains are plant-enriched or -depleted.

Synthetic communities display deterministic niche sorting in the plant microbiome

Plant roots and shoots sustained a higher bacterial alpha-diversity than did the agar (Figure 1a). Agar, root and shoot assembled distinct communities and fraction explained most of the variance in community composition between samples despite environmental perturbations (Figure 1b). Root and shoot enrichment, compared with the agar, closely followed phylogeny: 29/33 of Actinobacteria and 9/13 of Alphaproteobacteria were significantly enriched in the plant tissue, while 12/19 of Beta- and Gammaproteobacteria were plant depleted (Figure 2). 41 USeqs were differentially abundant in roots vs. shoots. Whereas in a typical natural survey, approximately 30% of OTUs shift significantly between root and soil [19], here 69 out of 97 USeqs were significantly enriched or depleted in plant tissue in at least one condition and the remaining 28 USeqs were also the rarest SynCom members. The fraction and stress variables in the GLM explained a cumulative 47% of the variation observed and the fraction:stress interaction explained an additional 19% of the variation (Table 1), indicating that in this system, plant colonization is largely deterministic.

Abiotic conditions correlate with the composition and diversity of the plant microbiome

Next, we asked how did the gradients of abiotic conditions influence the composition and diversity of the plant microbiome. Alpha-diversity of root and shoot communities were found to be positively correlated with Pi concentrations and temperature and negatively correlated with salinity (Figure 3). We used PERMANOVA tests on Bray-Curtis distances to quantify the effect of each of the environmental gradients on bacterial community composition, using a design matrix that includes all three fractions within each stress. In the measured ranges, temperature had the strongest effect on community composition ($P<0.001$, $R^2=0.41$), but Salinity, pH and Pi concentrations, had a significant effect as well (Pi: $P<0.001$, $R^2=0.09$; Salinity: $P<0.001$, $R^2=0.1$; pH: $P<0.001$, $R^2=0.06$). Using an NB-GLM, we found that 94 Useqs, exhibited a significant change in abundance in response to one or more environmental gradient.

We reasoned that strains exhibiting a differential response to stress across fractions (e.g increasing in abundance on planted agar whilst decreasing on unplanted agar), are responding to stress-induced changes in the plant. The abiotic conditions had a significant effect on the plant's ability to shape its microbiome. 54 USeqs were differentially enriched in different fractions depending on environmental conditions. This type of interaction suggests that these strains are not responding to abiotic conditions per se, but rather responding to changes in the plant niche resulting from these abiotic changes.

Co-occurring strains form distinct phylogenetic and functional guilds

We hypothesized that co-occurring strains occupy similar niches and thus act as functionally coherent units with respect to the plant. To test this, we classified the SynCom into four modules of co-occurring strains, based on pairwise correlation across all conditions and fractions. Due to the strong phylogenetic signal in plant colonization patterns, these modules were phylogenetically distinct: Module A contained Beta- and Gammaproteobacteria and is depleted in plant tissue compared with agar; module B contained mainly Firmicutes and is characterized by low abundance and no apparent enrichment/depletion pattern; modules C and D containing mainly Alphaproteobacteria and Actinobacteria, respectively, were both enriched in the plant tissue compared with the agar, but differ in response to stress. We inoculated A. thaliana seedlings with SynComs consisting of each of these modules in all possible combinations of 0, 1, 2, 3 and 4 modules (a sum of 16 combinations). In addition, we inoculated a cohort of plants with four mutually exclusive combinations of 50 strains with an equal representation for each module. We measured root and shoot sizes in response to each of the combinations.

The most pronounced morphometric effect was observed for main root elongation. Plants inoculated with modules C and D, containing plant-enriched strains, had a significantly shorter main root competed with inoculated plants and formed significantly more lateral roots. Conversely, plants inoculated with modules A and B, composed of plant-depleted isolates, had a weaker effect on root length and did not form more lateral roots than inoculated plants. Moreover, co-inoculation of module A with each one of the other modules, resulted in a morphology similar to inoculation with cluster A alone, indicating that module A is epistatic to modules C and D with respect to the plant's root morphology (Figure 4). These results provide direct evidence that interactions between components of the microbiome alter plant phenotypes.

Discussion

This study provides a systemic analysis of the influence of the abiotic environment on the assembly of plant microbiomes. We show that under controlled conditions, community assembly cues are robust and predictable across multiple abiotic gradients. Plant colonization patterns are largely driven by phylogeny, and colonization phenotypes are ubiquitous across different specimens of the same phylogenetic class. Subsets of the full synthetic community were shown to induce different root morphologies, and the morphology observed with the full community is an outcome of epistasis between two functional guilds.

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