Abstract

Plants respond to limiting concentrations of inorganic phosphate (Pi) in the environment by activating a phosphate starvation response (PSR). PSR encompasses a complex array of transcriptional responses, manifested in morphological, developmental, and physiological changes. As PSR was originally described under sterile conditions, the relevance of these findings to realistic environmental conditions is unknown because plant roots are intimately associated with microbial communities. These communities and their interactions with the plant can range from competition to cooperation depending, among other factors, on the nutrient content of the surrounding soil. In order to study the interplay between induction of PSR and plant-microbe interactions under limiting Pi conditions, we used a 'split-root' assay in which *Arabidopsis thaliana* roots were divided across two sides of an agar plate differing in Pi concentrations and in the inoculation of a 35-member synthetic bacterial community (SynCom). The induction of PSR in the plant was measured using a reporter system that expresses β-glucuronidase (GUS) under the control of the IPS1 promoter, which is strongly induced at low Pi. We found that high Pi in one compartment repressed bacterially-induced PSR in the adjacent low Pi compartment, indicating that regulation of signaling in bacterially-triggered PSR is systemic. PSR induced by bacteria has common regulatory and signaling elements with the canonical PSR that occurs in sterile conditions, and could serve as a model for studying the mechanisms of PSR induction in nature. Plants grown with bacteria increased Pi accumulation in Pi-replete media compared to plants grown in sterile conditions. We observed clear bacterial community shifts between the plant and the agar, and several strains significantly changed in relative abundance in response to direct and plant-mediated effects of Pi concentration, suggesting that systemic PSR-related signals are both induced by bacteria and affect them.

Introduction

Phosphorus is an essential macronutrient for the development of living organisms as a key component in fundamental macromolecules such as nucleic acids and phospholipids [1]. Phosphorus is often a limiting nutrient for plants because the chemically assimilable form, phosphate (Pi), is scarce and unevenly distributed in soils [1]. To obtain Pi, plants have developed a complex network of morphological, developmental, and physiological responses, collectively known as the Phosphate Starvation Response (PSR) [1]. This response is controlled

by a regulatory system activated by deficient Pi concentrations in the environment and/or inside plant cells [2][5].

Microorganisms form diverse communities in the area surrounding plant roots and shoots, referred to as the rhizosphere and phyllosphere, respectively, as well as within the plant's endophytic compartment (EC) [3]. Together, these communities are defined as the plant microbiome. While some plant species can mobilize nearby Pi resources by forming cluster roots that release exudates containing carboxylates, other species depend on microorganisms such as Mycorrhizas to support plant growth in Pi impoverished soils [12]. Beneficial plant-associated microbes have been shown to support and increase plant health and growth by providing phosphate and other nutrients to the host plant [12] and by providing pathogen defense through beneficial immune stimulation [10].

By activating PSR, plants increase Pi absorption. These responses can be either local, where a certain region in the root responds to the Pi conditions in its immediate vicinity, or systemic, where a local environment activates a long-distance response throughout the plant. Previous studies have indicated that plants activate local regulation when Pi is deficient in the local environment by altering the physiological characteristics of root growth in plants such as extending lateral roots in the soil to scavenge for Pi [7] and by reducing root cell size [8]. Plants activate systemic regulation to communicate between parts of the root system with unequal access to Pi [9], and this involves responses such as systemic repression of PSR if the shoot receives sufficient nutrients despite poor nutritional access in some areas of the root system [18]. However, former Pi starvation studies have yet to consider how microbial interactions can affect the type of regulation and response induced in low Pi concentrations. Since Pi is essential for

both microbial proliferation and plant growth, plant-microbe interactions are subject to contrasting selective pressures. The bacteria depend on the carbon secreted by the plant, but are also forced to compete with the plant for phosphate [3].

Understanding the relationship between PSR and the plant microbiome could potentially aid in tailoring the plant microbiome to be used to increase Pi accumulation, reduce the environmental burden of fertilizers, and therefore promote sustainable growth in crops. Previous experiments have shown that bacteria are required to induce activation of PSR in low Pi concentrations in the absence of sucrose, which is necessary to induce PSR under sterile conditions [6]. This finding indicates that bacterial communities play a role in inducing plant responses to the abiotic environment.

While the natural soil and root microbiome is difficult to replicate in artificial lab conditions, controlled settings can allow us to analyze relationships between PSR and microorganisms. *Arabidopsis thaliana* plants and their growth conditions can easily be manipulated experimentally due to the extensive knowledge the research community has built on this small model plant over the past 30 years [4]. In this study, we attempted to analyze whether bacterially-induced PSR is regulated in a systemic or local fashion in *A. thaliana* plants. We also asked whether systemic signals produced in the plant in response to different Pi concentrations influenced the composition of plant-associated bacterial communities. To answer these questions, we deployed a small bacterial synthetic community (SynCom) chosen to represent the dominant phyla present in wild plant-associated microbiomes in a split plate system. This system allowed us (a) to ensure that roots from the same plant could be divided and grown on different media conditions and (b) to separate the abiotic stress the plant is encountering from the stress

the bacteria encounter, thus disentangling the direct effect of the stress on the bacteria from indirect effects mediated by the plant.

Methods

Selection of the 35-members synthetic community

Bacterial community selection. We constructed a synthetic bacterial community by selecting 35 diverse bacterial strains isolated from roots of *Arabidopsis* and other *Brassicaceae* species (32 strains) and from a wild soil (2 strains) [16]. In addition, a non plant-associated microbe, *Escherichia coli* DH5α, was included as a control (Table 1). Each bacterial strain from the 35-member SynCom was selected from a larger isolate collection in a way that maximizes SynCom diversity and retains enough differences in their 16S rRNA gene to allow for easy and unambiguous identification (Figure 1a). This SynCom of taxonomically diverse and genome-sequenced bacteria approximates the phylum-level distribution observed in wild-type root endophytic compartments (Figure 1a) [16].

For SynCom experiments that used the GUS reporter gene for measuring phosphate starvation stress, two bacterial strains (MF41 and MF362) were removed from the full 35-member SynCom due to intrinsic GUS activity that would interfere with the assay, as demonstrated in preliminary experiments (Figure 1b).

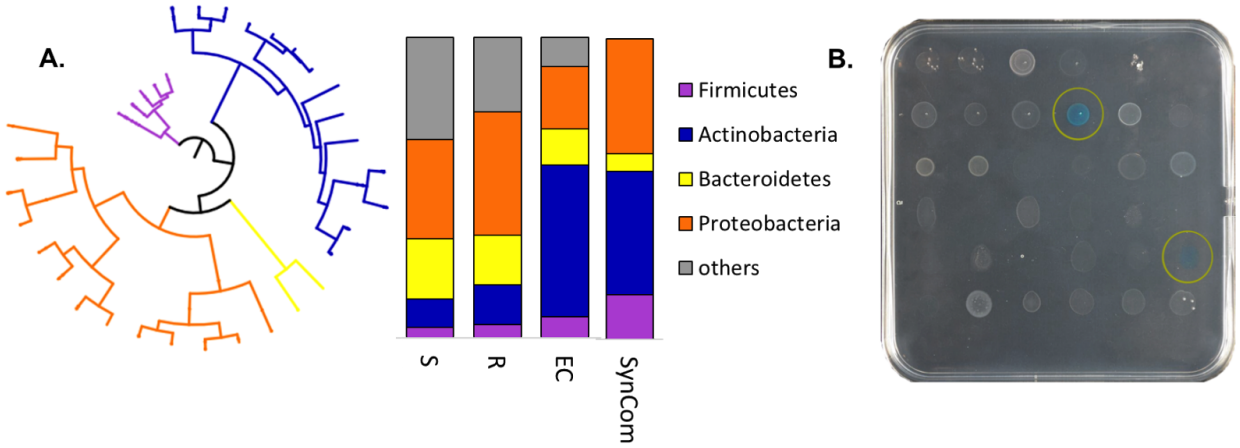

Figure 1. A) Phylogeny tree and taxonomy of the 35-member SynCom. SynCom taxonomy is representative of bacteria phyla of wild soil communities in shoots (S), roots (R), and the endophytic compartment (EC). B) MF41 and MF362 expressed GUS activity.

Bacteria growth conditions. Each strain of bacteria in the community was cultured by using a single colony to inoculate a test tube containing 4 mL of 2XYT medium (16 g/L Tryptone, 10 g/L Yeast Extract, 5 g/L NaCL). Liquid cultures were grown at 28˚C with agitation with the exception of *E. coli* grown at 37˚C. Cultures were centrifuged at 4000 g for 8 minutes to remove the media, and were washed in 10 mM $MgCl_2$. This process was repeated 3 times and the samples were resuspended at a final volume of 1 mL 10 mM $MgCl_2$. Bacterial concentrations were equalized according to optical density (OD_{600}) to a final concentration of 10^5 colony forming units (CFU) per mL of medium, assuming than an OD of one equals 10^9 CFUs/mL. SynComs were added into medium cooled to near solidification, and then mixed with agitation.

<u>Split root assay</u>

Seed sterilization. *A. thaliana* (Col-0) and the PSR reporter line *IPS1:GUS* [11] seeds were sterilized by agitating for 8 minutes in a solution containing 70% bleach with 0.2% Tween-20, and then washed 3x with sterile water.

Plant growth conditions. Seeds of both genotypes were germinated on Johnson medium [KNO_3 (0.6 g/L), $Ca(NO_3)_2 \cdot 4H_2O$ (0.9 g/L), $MgSO_4 \cdot 7H_2O$ (0.2 g/L), KCl (3.8 mg/L), H_3BO_3 (1.5 mg/L), $MnSO_4 \cdot H_2O$ (0.8 mg/L), $ZnSO_4 \cdot 7H_2O$ (0.6 mg/L), $CuSO_4 \cdot 5H_2O$ (0.1 mg/L), H_2MoO_4 (16.1 µg/L), $FeSO_4 \cdot 7H_2O$ (1.1 mg/L), Myo-Inositol (0.1 g/L), MES (0.5 g/L), pH 5.6 - 5.7] solidified with 1 % bacto-agar (BD, Difco) and supplemented with 0.5% sucrose and 1 mM Pi (KH_2PO_4) [16]. Depending on the experiment, filter sterilized Pi was added after autoclaving to Johnson medium at high concentrations of 1 mM Pi or at low concentrations of 25 µM Pi. Seeds were grown in two rows (about ~15 seeds per row) per square plate and plates were placed vertically in a growth chamber in a 15-h dark/9-h light regime (21 °C day /18 °C night). Seedlings germinated for 6 days before we induced split root formations.

Split root formation and transplanting. After 6 days of seed germinations, we stimulated lateral root formation by slicing the main root with a razor blade, and allowed the plants to grow for an additional week. Plants with split roots were then transferred to round petri plates with an impenetrable division in the middle. Plate conditions are described in the following section. When transferring, we carefully split lateral roots of each plant and placed half of the roots on either side without cross contamination; one plant shoot was placed on one side and the other shoot on the other side to control for shoot placement. Two plants were transferred to each plate.

Split root experimental design. Divided petri plates were used to split plant roots between two sides of the plates, exposing the plant to heterologous phosphate or bacterial conditions. We placed plant roots on 4 different environmental conditions made by Johnson medium without sucrose added. Roots were exposed to medium with added 1 mM Pi, 1 mM Pi + SynCom, 25 µM Pi, or 25 µM Pi + SynCom. Using the divided plates, we constructed homologous and

heterologous environments by varying combinations of the modified Johnson medium. The 10 possible environments created are listed in Table 2. Plants transferred to these different conditions were placed back into the growth chamber for another 7 days before Col-0 plants were harvested and *IPS1:GUS* plants were stained with GUS solution.

GUS staining and imaging. In order to visualize activation of PSR, we used transgenic *IPS1* construct (*IPS1:GUS*) plants containing the promoter for the gene *IPS1* merged with the coding region for the reporter line that expresses the enzyme β-glucuronidase (GUS) in the pBI101 binary vector [11]. Stressed plants overexpressing the PSR reporter construct *IPS1:GUS* [15], which is induced at low Pi, could be visualized after being stained with a GUS solution [$NaHPO_4$ (50 mM) pH 7.2, 0.5% Triton X-100, $C_{14}H_{13}BrClNO_7$ 'X-Gluc' (1mM)]. The IPS1 promoter drives GUS expression under phosphate starvation, creating a distinct phenotype from the addition of X-Gluc contained in the GUS solution. X-Gluc in the GUS solution reacted with the enzyme when incubated, and allowed roots under phosphate starvation to develop a blue root phenotype, making it easy to differentiate between roots that did or did not activate PSR. The split plate system combined with the GUS stain allowed us to analyze and differentiate between local and systemic PSR regulation. To stain, about 1 mL of the GUS solution was pipetted on top of medium and roots on each side of the plate, and then gently spread by rocking the plate, making sure the entire side was covered in the solution. Plates spread with the solution were incubated at 37 ˚C overnight and imaged the following day.

Determination of shoot phosphate concentration. The phosphate concentrations of the shoots of plants in the full member SynCom experiments were determined by the Ames method [13].

Extraction of DNA from roots and shoots. Roots and shoots were harvested to isolate bacterial DNA in the full member SynCom experiments. Roots from each plant were collected separately from both sides of the divided plate. Shoots were harvested from right and left sides of plates in each condition to control for shoot placement. To isolate bacterial DNA, harvested roots and shoots were rinsed 3 times with sterile distilled water to remove weak or non-plant associated bacteria and agar. Samples were then lyophilized, and stored at -80 ˚C for DNA extraction as described below.

Extraction of DNA from agar. To isolate bacteria from agar, a freeze and squeeze protocol was used [16]. A sterilized square of miracloth was placed at the bottom of 60 mL syringes, which were then filled with agar samples from the plates. We placed parafilm at the syringe tips and wrapped the plunger to the syringe before storing at -20 ˚C for a week. Syringes were then thawed at room temperature and liquid was squeezed from the agar into 50 mL tubes. Samples were pelleted by centrifugation for 20 min, supernatant was removed, and samples were centrifuged again in microfuge tubes to remove the remaining supernatant. The pellets were stored at -80 ˚C for DNA extraction.

DNA extraction.

DNA extractions were performed on root, shoot, and agar samples using 96-well format MoBio PowerSoil Kits (MOBIO Laboratories 2746 Loker Ave W #A, Carlsbad, CA 92010) following manufacturer's instructions.

16S sequencing. For SynCom bacterial 16S sequencing, we modified the Lundberg, D.S. *et al.* method [14] in library processing as described in Castrillo, G. *et al* [16]. We used 3 sets of index primers to amplify the V3-V4 regions of the bacterial 16S rRNA gene using primers 338F

(5'-ACTCCTACGGGAGGCAGCA-3') and 806R (5'-GGACTACHVGGGTWTCTAAT-3'). Primary PCR reactions were performed in triplicate and peptide nucleic acid (PNA) PCR blockers were used to reduce contamination by plant host plastid and mitochondrial 16S amplicons. PCR reactions were performed using the following specifications:

5 µL	Kapa Enhancer
5 µL	Kapa Buffer A
1.25 µL	5 µM 338F
1.25 µL	5 µM 806R
0.375 µL	mixed PNAs (1:1 mix of 100 µM pPNA and 100 µM mPNA)
0.5 µL	Kapa dNTPs
0.2 µL	Kapa Robust Taq
8 µL	dH_2O
5 µL	DNA

Temperature cycling

95 °C for 60 seconds
24 cycles of
    95 °C for 15 seconds
    78 °C (PNA) for 10 seconds
    50 °C for 30 seconds
    72 °C for 30 seconds
4 °C until use

PCR reactions were cleaned to remove primer dimers by using AMPure XP magnetic beads per manufacturer's protocol and then underwent a second, 9 cycle PCR step to add barcode primers indexed with the same reaction and 9 cycles of the cycling conditions described in Lundberg, D.S. *et al* [14]. The presence of PCR product was confirmed using gel electrophoresis. The samples were sequenced on an Illumina MiSeq instrument using a 600-cycle V3 chemistry kit.

Data analysis

SynCom sequencing data were processed with MT-Toolbox [14]. Categorizable reads (i.e. reads with correct primer sequences that successfully merged with their pair) were quality

filtered with Sickle by not allowing any window with Q-score under 20, and trimmed from the 5' end to a final length of 270 bp [16]. The resulting sequences were mapped to a reference set of the SynCom strains generated from Sanger sequences. A sequence from a contaminant strain (47Yellow) and Arabidopsis organellar sequences were added to the database [16]. 76% of sequences matched an expected isolate, and the sequence mapping results were used to produce an isolate abundance table.

Beta diversity was measured by calculating the Bray-Curtis index of community dissimilarity, and visualized using NMDS ordinations [17]. Strain co-occurrence was measured using Pearson correlation coefficients. Strain enrichment patterns were explored using a negative binomial generalized linear model implemented in the DESeq package in R [17].

Results

Pi concentration in the shoots correlates with the amount of Pi in the medium.

Previous studies have demonstrated that the activation of a systemic regulation response requires Pi internalization [1]. To confirm that the Pi accumulation in shoots was consistent with the Pi concentration in the media the plant was grown in, we measured the Pi accumulation in the shoots of plants grown on media with heterogenous (i.e. Pi added to only one side of the plate) and homogeneous (i.e. Pi added to both sides of the plate) Pi and bacterial concentrations. Pi accumulation was significantly decreased in the shoots of plants grown on sterile media with homogeneous low Pi concentrations compared to Pi accumulation in the shoots of plants grown on sterile media with homogeneous high Pi concentrations (Figure 2). Similar trends were noted in conditions with SynCom on both sides (Figure 2). In plants where the same root was split into two parts and supplied with media that differed in Pi concentrations, Pi accumulation was noted

to be less than Pi accumulation in plants grown under high Pi concentration and more than Pi accumulation in those grown in low Pi concentration (Figure 2). These results indicate a correlation between Pi concentration in the medium and Pi accumulation in the shoots.

Results also showed a stronger relative increase in Pi accumulation between plants grown in 25 µM Pi and 1 mM Pi in the presence of SynCom compared with sterile plants, indicating that bacteria enhanced the effect of Pi in the media on shoot Pi accumulation. However, this difference was not statistically significant.

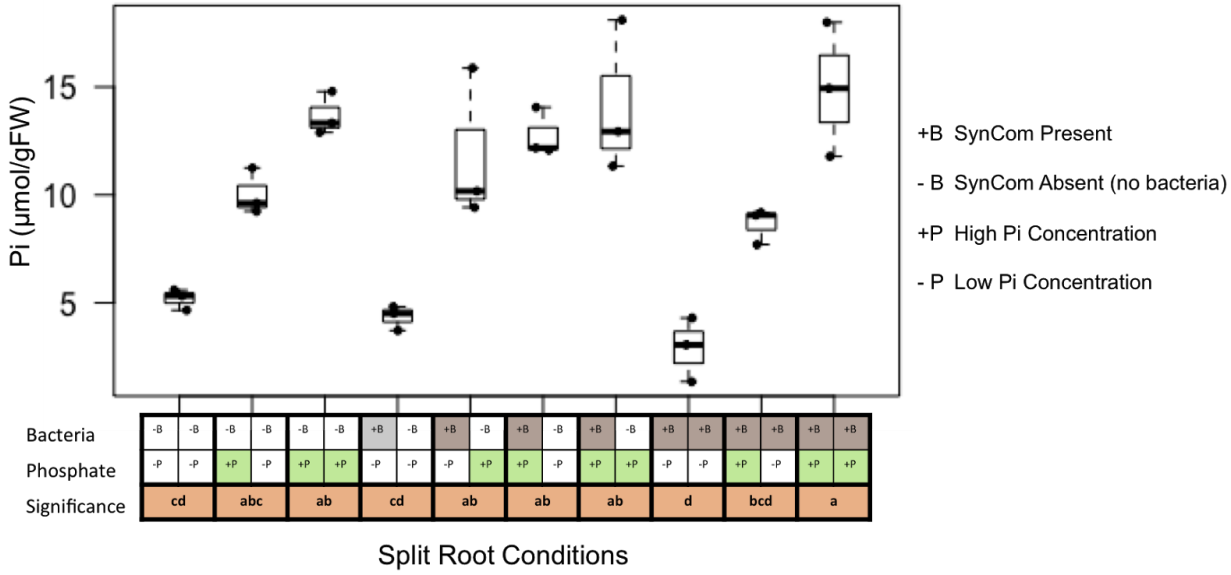

Figure 2. Boxplot of Pi accumulation in shoots of plants grown under heterogenous bacterial and Pi conditions. Pi accumulation in the shoot increases as the Pi concentration in the media increases. Table below the boxplot displays the media condition on each side of the split plates [SynCom present (+B), SynCom absent (-B), high Pi (+P), low Pi (-P)] as well as the significance results of the post-hoc analysis.

<u>PSR is systemically regulated in the presence of SynCom.</u>

In plants grown in split root conditions with no SynCom on either side, none of the 34 roots in each of the three varying Pi concentrations exhibited PSR on either side of the plate

(Figure 3a, 3b, 3c). As expected in these negative control conditions, plants grown in low Pi concentrations did not exhibit PSR in the absence of both bacteria and sucrose.

In plants grown on split root conditions with limiting Pi and SynCom present on both sides, 61 of 68 roots from both sides of the plate displayed Pi starvation induced GUS activity (Figure 3d). This positive control confirmed the presence of SynCom is able to induce PSR in limiting Pi conditions. Little GUS activity was observed in plants grown in homogenous SynCom and full Pi conditions (Figure 3f).

In roots grown with SynCom on both sides, high Pi in one side, and low Pi in the other, no GUS activity was detected in the high Pi compartment as expected. The Pi-starved roots also did not exhibit GUS activity, indicating a systemic repression was signalled from the roots in high Pi to the roots in low Pi (Figure 3e).

In plants grown on split root conditions with limiting Pi on both sides and SynCom on only one side, induction of GUS activity was seen on both sides (Figure 3g). This result could be interpreted as the result of bacteria moving into the uninoculated side through the root system. Little GUS activity was seen in the split root conditions with high Pi on both sides and SynCom on one side (Figure 3j). Plants grown with SynCom and low Pi on one side, and no bacteria and high Pi on the other showed a systemic repression of PSR on the side with low Pi (Figure 3i). Once again PSR was repressed in the SynCom side due to high phosphate received by the no SynCom side of the root system. Lastly, plants grown with SynCom and high Pi on one side, and no SynCom and low Pi on the other side showed little expression of GUS activity (Figure 3h). The GUS activity was observed to be more prevalent in the axenic sides of the divided roots. Results were reproduced in three replicates.

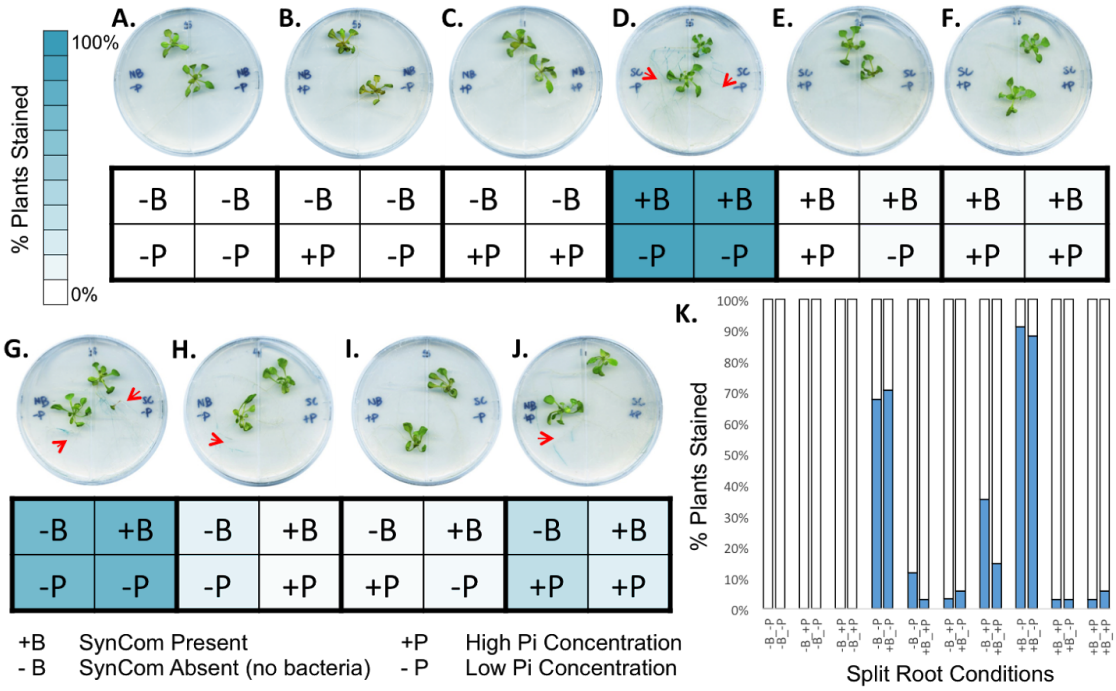

Figure 3. A-J) GUS stain plates under different SynCom and Pi conditions. Each plate represents a unique split plate condition totaling 10 possible combinations of Pi and SynCom. Table below the plates describes the media condition on each side of the plate, and percentage of plants expressing GUS activity is illustrated by blue shading. K) Bar graph of the percentage of plants (n=34) displaying the blue root phenotype. GUS activity was visualized in each condition in three replicates.

Bacterial colonization

High throughput sequencing of DNA extractions was used to determine the presence and abundance of each bacteria from the community in the root, shoot and agar. The DNA sequences were mapped to each member of the bacterial community, and revealed 4 contaminants as well as 24% unmapped sequence reads. Plates containing no bacteria did not show visual signs of contamination, while embedded bacteria were visible in the media supplemented with the

SynCom. Comparisons of the relative abundance of mapped reads reveal a clear differentiation between bacterial communities formed in agar and plant fractions (Figure 4a, 4b).

We observed a separation between the root and shoot communities as well. We were able to conclusively state a community shift occurred between plant and agar environments from the original SynCom. The community separations between the root and agar were primarily due to strains in the Actinobacteria and Proteobacteria phyla, while Firmicutes did not contribute as much to the community differences. 8 of 10 Actinobacteria, 1 of 2 Bacteroidetes, 1 of 5 Firmicutes, and 6 of 15 Proteobacteria were significantly enriched in the root over the agar (Figure 4a, 4c). Interestingly, 6 of 7 strains that were significantly depleted in the root compared to the agar were Proteobacteria, suggesting the strains are either prolific in the agar or the strains do not colonize the roots as well as other strains (Figure 4a, 4c). These results suggest closely related bacteria in the phylogentic tree had similar colonization patterns.

We did not observe separate NMDS clusters when comparing different Pi conditions (Figure 4b). In addition, a comparison of the communities in the same SynCom conditions and differing Pi conditions revealed an enrichment in only 2 strains in the agar, MF161 and MF345, both of which belong to the Actinomycetales order (Table 1). MF345 was also enriched in low Pi conditions in the roots. These results suggest that the bacterial community is not strongly affected by the Pi content of the media. To test the indirect or systemic effect of Pi on bacteria, we compared the community composition in split plate conditions with the same Pi condition on one side, but differing on the other. We noticed that a community shift occurred in the agar (but not in plant), mainly from an enrichment of Actinobacteria, in communities under full Pi resources locally and low Pi conditions across the barrier (Figure 4c). Together, these results

indicate that bacterial communities may be more sensitive to plant-mediated changes to their environment induced by Pi starvation, rather to the Pi starvation itself.

When comparing community differences between split plate sides with SynCom and sides without SynCom, we observed that there were bacteria on the uninoculated side of the plate (Figure 4c). This suggests that certain bacteria were able to travel through the root system. The side of the plates that did not have bacteria added were colonized with bacteria from the inoculated side, likely via the plant itself, as the root bridges the physical barrier. We also noticed that the agar fraction formed NMDS clusters based on samples collected from media supplemented with or without the SynCom (squares vs triangles in Figure 4a).

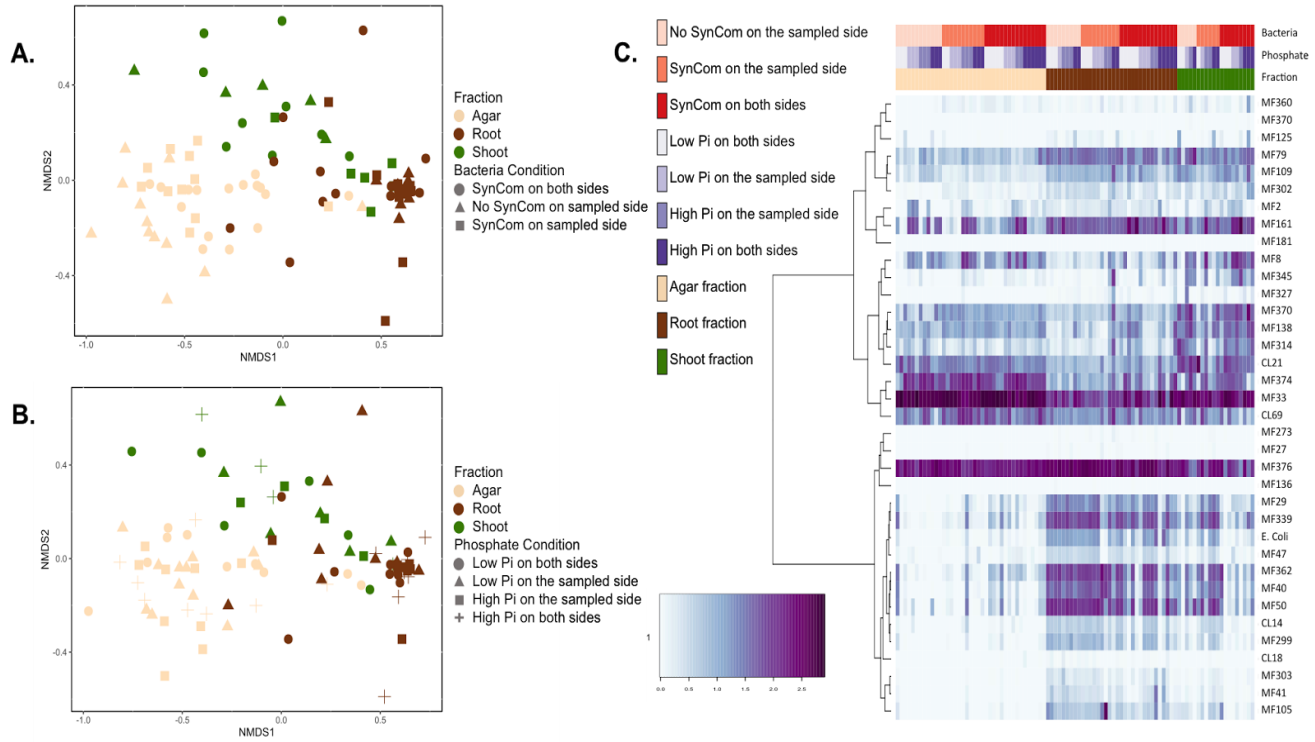

Figure 4. A) NMDS graph of bacterial communities clustering based on fraction of the plant sampled and on presence of SynCom in agar. B) NMDS graph of bacterial communities clustering based on fraction of the plant

sampled, but not based on Pi concentration. C) Heat map of bacteria mapped show bacterial communities organized by fraction sample, condition grown in, and phylogeny.

Discussion

In this study, we demonstrated that the Phosphate Starvation Response is systemically regulated in the presence of a 35-member synthetic bacterial community by performing a split root assay and visualizing stress response with GUS staining. Our results suggest that bacteria can trigger plants to signal long distance when part of its root system has access to essential nutrients. For example, in the presence of microbes, roots grown in limiting Pi conditions did not exhibit a PSR phenotype when another portion of the root system was grown in high Pi conditions, illustrating a systemic repression of PSR activation. We observed clear bacterial community shifts between the agar and the plant, but only a few of the strains were significantly affected by the phosphate conditions. Interestingly, a strong shift in the agar community in high Pi conditions occurred when the original SynCom was subjected to indirect low Pi conditions, indicating a possible systemic effect from plant, possibly via changes in exudate composition. Gaining a better understanding of the role plant microbiomes play in plant regulation processes under environmental stress can allow us to manipulate microbiomes to facilitate plant nutrient acquisition.

Current efforts are focused on optimizing bacterial colonization experiments to determine whether bacterial community compositions change in conjunction with the plant's regulation of PSR. Optimization is still needed in these colonization experiments, as MiSeq data has had contamination and large amounts of reads that could not be mapped to the input database. Contamination could be caused during DNA extractions and amplification, but could also indicate that the sterilized seeds contain some bacterial DNA. In addition, the 35-member

SynCom may not be suitable for testing and analyzing community changes; a larger and more diverse SynCom representative of wild root microbiome communities would be needed to better understand changes in the microbial community in response to PSR activation.

Future directions involve further confirmation and validation of GUS stain results by performing qPCR on the RNA in collected root samples to analyze which phosphate stressed genes are amplified, and therefore activated under different bacteria and Pi conditions. The methods developed in this study contribute to continued efforts to identify and characterize plant regulatory mechanisms under environmental stress in nature. PSR triggered by SynCom has common regulatory and signaling elements with the canonical PSR that occurs in sterile conditions, and offers a model to study novel PSR induction pathways. In conclusion, split root and synthetic community experiments could be conducted on other essential plant nutrients such as nitrogen, and strengthen our understanding of how plants respond to uneven nutrient distribution in wild soils amongst wild root microbiome communities.

Appendix I.

Table 1. 35-member synthetic bacterial community.

Isolate #	IMG taxon_oid	Phylum	Class	Order	Family	Genus	Source
MF105	2517572206	*Firmicutes*	*Bacilli*	*Bacillales*	*Bacillaceae*	*Bacillus*	Arabidopsis thaliana
MF125	2561511073	*Firmicutes*	*Bacilli*	*Bacillales*	*Bacillaceae*	*Bacillus*	Capsella rubella
MF27	2522125133	*Firmicutes*	*Bacilli*	*Bacillales*	*Bacillaceae*	*Bacillus*	Arabidopsis thaliana
MF41	2563366514	*Firmicutes*	*Bacilli*	*Bacillales*	*Bacillaceae*	*Bacillus*	Soil
MF370	2643221500	*Proteobacteria*	*Alphaproteobacteria*	*Rhizobiales*	*Brucellaceae*	*Ochrobactrum*	Cardamine hirsuta
CL21	2558309150	*Proteobacteria*	*Betaproteobacteria*	*Burkholderiales*	*Burkholderiaceae*	*Ralstonia*	Arabidopsis thaliana
MF376	2521172625	*Proteobacteria*	*Betaproteobacteria*	*Burkholderiales*	*Burkholderiaceae*	*Burkholderia*	Arabidopsis thaliana
MF374	2596583649	*Proteobacteria*	*Alphaproteobacteria*	*Caulobacterales*	*Caulobacteraceae*	*Brevundimonas*	Arabidopsis thaliana
CL14	2643221508	*Proteobacteria*	*Betaproteobacteria*	*Burkholderiales*	*Comamonadaceae*	*Variovorax*	Arabidopsis thaliana
MF47	2636416056	*Proteobacteria*	*Betaproteobacteria*	*Burkholderiales*	*Comamonadaceae*	*Polaromonas*	Arabidopsis thaliana
E.Coli		*Proteobacteria*	*Gammaproteobacteria*	*Enterobacteriales*	*Enterobacteriaceae*	*Escherichia*	
MF40	2563366720	*Bacteroidetes*	*Flavobacteriia*	*Flavobacteriales*	*Flavobacteriaceae*	*Flavobacterium*	Soil
MF8	2529292577	*Bacteroidetes*	*Flavobacteriia*	*Flavobacteriales*	*Flavobacteriaceae*	*Chryseobacterium*	Arabidopsis thaliana
MF273	2522125155	*Actinobacteria*	*Actinobacteria*	*Actinomycetales*	*Intrasporangiaceae*	*Terracoccus*	Arabidopsis thaliana
MF109	2522572063	*Actinobacteria*	*Actinobacteria*	*Actinomycetales*	*Microbacteriaceae*	*Leifsonia*	Arabidopsis thaliana
MF314	2521172612	*Actinobacteria*	*Actinobacteria*	*Actinomycetales*	*Microbacteriaceae*	*Curtobacterium*	Cardamine hirsuta
MF161	2517572124	*Actinobacteria*	*Actinobacteria*	*Actinomycetales*	*Micrococcaceae*	*Arthrobacter*	Arabidopsis thaliana
MF362	2563366511	*Actinobacteria*	*Actinobacteria*	*Actinomycetales*	*Micrococcaceae*	*Arthrobacter*	Arabidopsis thaliana
CL69	2593339129	*Proteobacteria*	*Gammaproteobacteria*	*Pseudomonadales*	*Moraxellaceae*	*Acinetobacter*	Arabidopsis thaliana
MF360	2521172630	*Actinobacteria*	*Actinobacteria*	*Actinomycetales*	*Mycobacteriaceae*	*Mycobacterium*	Arabidopsis thaliana
MF29	2519899643	*Actinobacteria*	*Actinobacteria*	*Actinomycetales*	*Nocardiaceae*	*Rhodococcus*	Arabidopsis thaliana
MF339	2643221496	*Actinobacteria*	*Actinobacteria*	*Corynebacteriales*	*Nocardiaceae*	*Rhodococcus*	Arabidopsis thaliana
MF345	2582580751	*Actinobacteria*	*Actinobacteria*	*Actinomycetales*	*Nocardioidaceae*	*Nocardioides*	Arabidopsis thaliana
MF181	2639762524	*Firmicutes*	*Bacilli*	*Bacillales*	*Paenibacillaceae*	*Paenibacillus*	Arabidopsis thaliana
MF302	2563366739	*Proteobacteria*	*Alphaproteobacteria*	*Rhizobiales*	*Phyllobacteriaceae*	*Phyllobacterium*	Arabidopsis thaliana
MF327	2522572130	*Actinobacteria*	*Actinobacteria*	*Actinomycetales*	*Promicromonosporaceae*	*Promicromonospora*	Arabidopsis thaliana
MF50	2228664007	*Proteobacteria*	*Gammaproteobacteria*	*Pseudomonadales*	*Pseudomonadaceae*	*Pseudomonas*	Arabidopsis thaliana
MF2	2517572231	*Proteobacteria*	*Alphaproteobacteria*	*Rhizobiales*	*Rhizobiaceae*	*Rhizobium*	Arabidopsis thaliana
MF33	2561511224	*Proteobacteria*	*Alphaproteobacteria*	*Rhizobiales*	*Rhizobiaceae*	*Agrobacterium*	Thlaspi arvense
CL18	2563366515	*Actinobacteria*	*Actinobacteria*	*Actinomycetales*	*Streptomycetaceae*	*Streptomyces*	Arabidopsis thaliana

MF136	2636416059	*Actinobacteria*	*Actinobacteria*	*Actinomycetales*	*Streptomycetaceae*	*Streptomyces*	Arabidopsis thaliana
MF299	2521172643	*Actinobacteria*	*Actinobacteria*	*Actinomycetales*	*Streptomycetaceae*	*Streptomyces*	Cardamine hirsuta
MF303	2521172626	*Actinobacteria*	*Actinobacteria*	*Actinomycetales*	*Streptomycetaceae*	*Streptomyces*	Arabidopsis thaliana
MF138	2593339266	*Proteobacteria*	*Gammaproteobacteria*	*Xanthomonadales*	*Xanthomonadaceae*	*Luteibacter*	Arabidopsis thaliana
MF79	2556921674	*Proteobacteria*	*Gammaproteobacteria*	*Xanthomonadales*	*Xanthomonadaceae*	*Dyella*	Arabidopsis thaliana

Table 2. Conditions with varying phosphate and bacteria.

Condition	left_bac	left_Pi	right_bac	right_Pi
A	NB	-P	NB	-P
B	NB	+P	NB	-P
C	NB	+P	NB	+P
D	SC	-P	SC	-P
E	SC	+P	SC	-P
F	SC	+P	SC	+P
G	NB	-P	SC	-P
H	NB	-P	SC	+P
I	NB	+P	SC	-P
J	NB	+P	SC	+P

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