IMPACT OF PHYLLOSPHERE *METHYLOBACTERIUM* ON HOST RICE LANDRACES

Short title: *Methylobacterium* increases rice yield

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Abstract

The genus *Methylobacterium* includes widespread plant-associated bacteria that dominate the plant phyllosphere (occupying leaf surfaces), consume plant-secreted methanol, and can produce plant growth promoting metabolites. However, despite the potential to increase agricultural productivity, their impact on host fitness in the natural environment is relatively poorly understood. Here, we conducted field experiments with three traditionally cultivated rice landraces from north eastern India. We inoculated seedlings with native vs. non-native phyllosphere *Methylobacterium* strains, finding significant impacts on plant growth and grain yield. However, these effects were variable: whereas some *Methylobacterium* isolates were beneficial for their host, others had no impact or were no more beneficial than the bacterial growth medium. Host plant benefits were not consistently associated with *Methylobacterium* colonization, altered phyllosphere microbiome composition, changes in early expression of plant stress response pathways, or bacterial auxin production. We provide the first clear demonstration of the benefits of phyllosphere *Methylobacterium* for rice yield under field conditions, and highlight the need for further analysis to understand the mechanisms underlying these benefits. Given that the host landrace-*Methylobacterium* relationship is not generalizable, future agricultural applications will require careful testing to identify coevolved host-bacterium pairs that may enhance the productivity of high-value rice varieties.

Keywords

Host-bacterial interaction, host fitness, phyllosphere, epiphytes, grain yield, rice landraces

Introduction

Plants associate with a diverse set of microbes, both belowground (“rhizosphere”) and aboveground (“phyllosphere”). The phyllosphere harbours nearly 10^7 bacterial cells/cm² of leaf surface, with both biotic and abiotic factors influencing the composition of bacterial communities [1,2]. The importance of phyllosphere microbes is highlighted by their large-scale impacts; for instance, leaf microbiome diversity contributes significantly to the productivity of tree communities [3]. Hence, a large body of work has focused on understanding the establishment and stability of phyllosphere communities as well as the interactions between community members and host plants. For example, in both wild and domesticated plants, microbial communities tend to be hierarchically structured. Soil microbiomes harbour maximum diversity and complexity, followed by rhizosphere and phyllosphere epiphytes that colonize leaf and stem surfaces, and finally, phyllosphere endophytes that colonize internal leaf tissues [4–8]. An important factor driving microbial assembly in the phyllosphere is the strength and nature of selection acting at the leaf surface. For instance, phyllosphere bacteria must deal with both antimicrobials and limited nutrients in plant leaf exudates, and exposure to UV radiation and desiccation [1,2]. These factors may impose selection for bacterial traits such as aggregation, deployment of efflux pumps, and the production of protective pigments and biosurfactants. Indeed, the composition of phyllosphere bacterial communities – which are typically dominated by Alphaproteobacteria and Gammaproteobacteria [9] – suggests a role for selection, rather than stochastic community assembly. However, the nature, strength and variability of such selection remains poorly understood.

Recent work does show broad potentially useful functions provided by phyllosphere bacteria, including nitrogen fixation [4,10,11]. However, whether and how these functions directly benefit the host plant is not well understood in most cases. Some of the best examples of direct fitness benefits to the host derive from studies on abundant phyllosphere bacteria from the genus *Methylobacterium*. This group includes species that consume methanol released by plant cell walls, such that methanol metabolism is key during phyllosphere colonization [7]. Many species from this group are thought to enhance host plant fitness [12,13]. For example, epiphytic *Methylobacterium* inoculation promotes moss seed growth to a level comparable to the effects of applying synthetic cytokinin [14]. Similar results are observed with cell-free culture suspensions of *Methylobacterium* growth media, which increase germination and seedling growth in wheat, potentially due to cytokinins secreted by the bacteria [15]. Some *Methylobacterium* strains enhance seed ripening in rice, though they have no impact on barley yield [16]. In fact, many *Methylobacterium* strains produce (or have the necessary genes or metabolic pathways for) a number of plant growth promoting factors such as cytokinins, that could confer significant fitness benefits for the host plant [12,13,17]. However, prior studies have largely been conducted in the laboratory or greenhouse, where the natural environmental context is missing; and it is therefore unclear whether the observed impacts of *Methylobacterium* can be extrapolated to field conditions. Furthermore, many studies use standard plant model systems to test the impact of bacteria isolated from various sources. To fully understand and use plant-microbe relationships, we must evaluate them in the field while maintaining the context of specific naturally-evolved associations.

Here, we tested the specificity of host-microbial relationships in three traditionally cultivated rice landraces from north east India. In this region, distinct rice landraces have been cultivated locally for many generations, and show substantial genotypic and phenotypic divergence [18]. Previously, we showed that host rice landrace was the best predictor of phenotypic variation in epiphytic *Methylobacterium* strains, with different landraces associating with distinct *Methylobacterium* strains [19]. Preliminary greenhouse experiments further showed that some rice landraces gain an early growth advantage when inoculated with *Methylobacterium* strains isolated from their leaves. Therefore, we hypothesized that *Methylobacterium* strains may confer a fitness advantage to their specific host plants during rice cultivation. To test this, we chose three phenotypically distinct landraces from our previous study, that are cultivated in the state of Manipur. We inoculated rice seedlings with a native *Methylobacterium* strain isolated from the specific landrace (“own” bacteria), or a non-native strain from a different landrace (“other” bacteria); and measured plant growth and yield related traits in an experimental field plot in Manipur (Fig. 1). Local adaptation between bacteria and hosts would result in host-specific fitness benefits, with potential implications for increasing agricultural productivity of the landraces. We find that the impacts of phyllosphere *Methylobacterium* varied across landraces: while some *Methylobacterium* strains were beneficial for their host plant, others either had no impact or were as beneficial as the bacterial growth medium control. In contrast, inoculating plants with the non-native *Methylobacterium* strain generally reduced plant fitness. Our results suggest that understanding locally evolved plant-microbial relationships in the phyllosphere is critical for developing agricultural applications.

Materials and methods

Bacterial strains and culture conditions

In 2016, we had isolated hundreds of *Methylobacterium* strains from the phyllosphere of multiple rice landraces from north east India [19]. In this study, we analysed the impact of the association of four of these strains on host rice plants in the field, choosing strains that were dominant in the respective host landrace. Three isolates were associated with rice varieties grown in the state of Manipur: strain CKPL1 (GenBank accession no. MN982816) from rice landrace Chakhao, strain PML2 (GenBank accession no. MN982833) from Phouren-mubi, and PNL1 (GenBank accession no. MN982835) from Phou-ngang. The fourth, DKS6 (GenBank accession no. MN982770), was isolated from Deku, a rice landrace from Arunachal Pradesh. Based on 16S rRNA sequencing, CKPL1 appears to be closely related to *Methylobacterium komagate*; PML2 to *Methylobacterium radiotolerans*, PNL1 and DKS6 to *Methylobacterium salsuginis* (the latter two strains are now reclassified as the genus *Methylobacterium*) [20]. To prepare bacterial inoculum, we inoculated 40 μ L of *Methylobacterium* glycerol stocks in 300 mL Hypho supplemented with 60 mM methanol and 2 mM succinate as the carbon source; and allowed growth for 48 hrs at 30°C in a shaking incubator (see S1 Table for media composition; [21]. The final OD_{600nm} ranged from 1-1.3 depending on the growth of the *Methylobacterium* strain used.

Field experiment

We identified a plot of farmland in Chingarel village of the Imphal East district of Manipur, a part of a larger area cultivated by a local farmer. Usually, the farmer cultivates 3-5 different rice varieties simultaneously in the same field. However, in

2018 the farmer decided to only plant a commercially available high yielding rice variety. We designed our experimental plot such that it was not close to these plants (S1A Fig). The experiment was conducted from June to December 2018 (Fig. 1), coinciding with the usual rice growing season in this region (Fig. 1A). A month before planting, the field was prepared as usual, using a hoe to plough the soil and raising mud boundaries to separate the treatment blocks; and without the addition of any fertilizer to the experimental plot (S1A Fig).

We obtained seeds of the three experimental rice landraces (Chakhao, Phourenmubi and Phou-ngang) from the preceding year's harvest, from the same farmer. For each landrace, we divided the seeds into four treatment groups: (i) Untreated, treated only with water, (ii) Broth control, treated with sterile bacterial growth medium, (iii) "Own", treated with the respective native *Methylobacterium* strain isolated from the landrace, and (iv) "Other", treated with the non-native *Methylobacterium* strain from Arunachal Pradesh. To ensure that we used viable seeds, we used excess seeds and allowed them to germinate for 4 days while wrapped in a wet cloth. We prepared the bacterial cultures and soaked only germinated seeds in their respective inoculum for 48 hrs (for instance, 300 ml culture for 300 germinated seedlings in a flask; S1B Fig). We then transplanted the soaked seedlings into the field, placing 4 seedlings together to constitute one "plant", and using a wooden frame with 1 ft x 1 ft squares to ensure sufficient spacing between plants (S1C Fig). For each landrace and treatment combination, we transplanted 30 plants; but subsequently we analysed only 15 of them (Fig. 1B) to minimize the impacts of cross-contamination across neighbouring plants. These rice landraces are typically cultivated as follows: seedlings are transplanted in wet soil, and then the field is allowed to flood under natural rainfall (wetland cultivation) until the ripening

stage. However, in 2018 Manipur faced a severe drought, with very little rainfall during the plants' reproductive phase (October onwards). To mitigate drought stress, we irrigated the experimental plot with purchased water in late October. All farming operations except the bacterial treatments were carried out by the farmer.

Forty-five days after transplantation of seedlings, we sprayed each rice plant with 10 ml of fresh inoculum (water, broth control, or broth with own or other *Methylobacterium* strains) (Fig. 1A). To test whether the sprayed bacteria successfully colonized the plants, we collected flag leaves from three plants per treatment, before and after spraying. We isolated *Methylobacterium* by imprinting the leaves on Hypho minimal agar with 0.5% methanol, and identified the strains by sequencing the 16S rRNA gene as described earlier [19]. As the rice plants grew, we measured traits such as plant height, flag leaf width and length, and the number of tillers and panicles [22] at three different timepoints (Fig. 1A). Finally, in early December, we collected all the grains produced by each individual plant and transported them to the laboratory in airtight plastic bags to measure the total number of grains and total grain weight per plant [22].

Determining phyllosphere microbiome composition

To determine the phyllosphere microbiome as a function of experimental treatments, we collected flag leaf samples at the seed ripening stage in early December (n = 2-6 flag leaves/plant/treatment/landrace). To obtain epiphytic bacteria from the leaf surface, we used a previous protocol with some modifications [23]. Briefly, we placed the cut leaf (3-4 cm length) into 1 ml of 1X Redford buffer (1M tris HCl; 0.5M EDTA; 1.24% triton) for 5 hrs with periodical vortexing. We removed the leaf tissue and

used the supernatant (containing bacteria dislodged from the leaf surface) for DNA extraction using a Qiagen DNeasy blood and tissue kit, with slight modifications to the standard bacterial protocol as follows. We centrifuged the leaf wash at 10000 rpm for 25 mins. To the pellet, we added 20 μ L proteinase K for 10-15 mins at 56°C and added 200 μ L of lysis buffer at 56°C for 2 hrs. After this, we followed the manufacturer's protocol without modification. We quantified the DNA in each sample using the Qubit 3 fluorometer (Invitrogen, ThermoFisher Scientific Inc.). Then, we amplified the V3-V4 hypervariable regions of the 16S rRNA using standard Illumina primers:

F5'TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGCCTACGGGNGGCWGCAG
3',

R5'GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGGACTACHVGGGTATCTA
ATCC3' (underlined bases indicate the adapter overhang). We blocked amplification of plant mitochondrial and chloroplast DNA by adding PNAs (polypeptide nucleic acid) in our 16S PCR reaction, as described previously [24]. We sequenced amplicons on the Illumina Miseq platform (300 x 2 paired end reads).

To analyse the microbiome, we used the DADA2 workflow to filter out reads with low quality ($Q < 30$) and generate ASV (amplicon sequence variant) tables [25]. Briefly, reads with $\geq 97\%$ sequence identity were retained and assigned taxonomy using the RDP (training set_14) and Silva reference databases (training set v123) [26,27]. To determine successful colonization by own and other *Methylobacterium* strains, we binned all *Methylobacterium* into three groups: identical or very similar (at least 97% sequence identity) to the experimentally inoculated "own" or "other" strains; or "environmental" *Methylobacterium* (*Methylobacterium* ASVs that were not classified

as own or other, and were therefore likely acquired from the environment). We calculated the relative abundance of each group, either as a fraction of the entire microbial community (i.e. all microbial reads) or as a fraction of all *Methylobacterium* reads. Similarly, we determined the relative abundance of the most common rice pathogens (*Burkholderia*, *Pseudomonas*, and *Xanthomonas*) [28,29] using sequence identity (100%) to the 16S rRNA sequences from the NCBI database (GenBank accession nos. MN400211.1, FJ151352.1, HM747119.1 respectively).

Greenhouse experiment to test the impact of *Methylobacterium* on gene expression in host seedlings

We tested whether inoculation of *Methylobacterium* leads to a change in PAMP (pathogen-associated molecular pattern) triggered immunity genes or stress related genes in the early stages of the host plant. We conducted a greenhouse experiment using the same inoculation protocols as described for the field experiment, but focusing on the rice landrace Chakhao. Based on earlier studies, we chose representative host genes that are important for host responses to various stresses, including the membrane receptor kinases *OsXA21* (specific to *XOO* factor of *Xanthomonas*; [29]) and *OsFLS2* (specific to flagella, [30]); the mitogen activated protein kinase *OsMAPK3/6* (involved in plant defence, [31]); transcription factors *OsWRKY13,53,62,76* (involved in plant defence; [31]) and *SNAC1* (drought stress; [32]); pathogenesis related proteins *PR1b*, *PR1-11*, *PR1-74*, *PR8* [33]; and genes involved in the production of secondary metabolites like *OsPAL1,4* (lignin biosynthesis, [34]) *OsACO1* (ethylene regulation, [35]) and *OsG1* (involved in plant defence and development, [36]).

After soaking seedlings in the respective bacterial cultures (or controls), we transplanted them into small pots (2 germinated seedlings per pot) maintained under flooding. We grew all plants in the greenhouse under natural light with 70% relative humidity. At four different time points (immediately before and after soaking seedlings in bacterial inoculum; and before and 24 hrs after spraying young plants with inoculum), we collected three replicate tissue samples (three seedlings or leaves from three plants) for each treatment (S2 Fig) and stored them at -80°C . We extracted total RNA from a single seedling or leaf using Trizol (Invitrogen) as per the manufacturer's instruction. We used 2 μg RNA for cDNA synthesis using a RevertAid cDNA synthesis kit (ThermoFisher) and measured gene expression using qRT-PCR (BioRad CFX system). For each biological replicate, we tested three technical replicates and normalized CT values for each target gene against *OsACTIN1*. Primers used for amplification are listed in S2 Table . We then calculated the normalized expression of each gene as $2^{-\Delta\text{Ct}}$, where $\Delta\text{Ct} = \text{Ct}_{(\text{gene})} - \text{Ct}_{(\text{actin})}$.

Testing auxin production by focal *Methylobacterium* strains

We tested whether our four *Methylobacterium* strains differed in their ability to produce auxin (IAA, indole acetic acid), using the Salkowski colorimetric assay [37]. We grew each strain (5 μL inoculum from glycerol stocks) in 20 mL Hypho broth supplemented with either 25 mM methanol, 25 mM methanol with 2 mM succinate, or 2 mM succinate alone (3 independently inoculated tubes per strain). We added 5 mM L-tryptophan to the growth media (substrate for IAA production) and incubated cultures at 28°C with shaking at 150 rpm for 5 days. We measured $\text{OD}_{600\text{nm}}$ of each culture and diluted with fresh sterile Hypho as necessary, so that all cultures had a similar cell density. After centrifugation at 10,000 rpm for 10 mins, we took 1 mL of

the supernatant, added 1 mL of Salkowski reagent, and incubated for 1 hr in the dark at room temperature. We measured IAA production by measuring color production at 530 nm (spectrophotometer Hitachi UH5300), and estimated the amount of IAA produced using a standard curve made from serial dilutions of synthetic IAA (Himedia) in Hypho broth.

Statistical analysis

We analysed and visualized data using R [38]. For all plant traits, we detected potential influential points using the function `influential.measures` in base R, and we report statistical analysis both with and without these influential points. We used two-way ANOVAs to test the effect of treatment and host landrace on each trait, using a generalized linear model (GLM) with Tukey's honest significant difference (Tukey's HSD) to account for multiple comparisons in the R package 'multcomp' [39].

To test the impact of bacterial treatment on the phyllosphere microbiome, we used PERMANOVA (Permutational analysis of variance) using the function 'Adonis' in the package 'Vegan' [40]. To visualize sample clustering based on bacterial community composition, we calculated Bray-Curtis distances between samples and performed Canonical Analysis of Principal Coordinates based on Discriminant Analysis (CAPdiscrim) [41] using the R package 'Biodiversity R' [42]. We tested for significant clustering and estimated classification success by permuting the distance matrix 1000 times, and estimating the probability of finding the observed differences by chance. We plotted the two dominant linear discriminants (LD) to visualize clusters. For each cluster, we drew ellipses reflecting 95% confidence intervals using the function 'Ordiellipse' in the R package 'Vegan' [40].

For the qRT-PCR data, for normalized expression levels of each gene, we used generalized linear models (GLM) with Tukey's honest significant difference (Tukey's HSD) to account for multiple comparisons. We carried out ANOVAs to test the effect of time and treatment on gene expression. For visualization of the datasets, we used the package 'ggplot2' [43].

Results

To test the impact of *Methylobacterium* on host rice plants, we designed field experiments incorporating four treatments in three rice landraces (Chakhao, Phouren-mubi and Phou-ngang) from Manipur (Fig. 1). "Untreated" seedlings were exposed to water before transplanting in soil and during foliar spraying; "Broth control" seedlings were exposed to sterile bacterial growth media; "Own" seedlings were exposed to a growing culture of native *Methylobacterium* that was originally isolated from the flag leaves of the specific landrace; and "Other" seedlings were exposed to a *Methylobacterium* strain that was originally isolated from a landrace from Arunachal Pradesh (Fig. 1). Based on potential interactions between the rice landraces and *Methylobacterium* strains, we outlined several possible outcomes and implications of the field experiment.

1. *Methylobacterium* does not increase host fitness relative to the broth control, in any landrace (Fig. 2A).
2. *Methylobacterium* is only beneficial to its "own" landrace. In this case, we expected the fitness of "own" plants to be higher than that of "broth control"

plants; but the fitness with “other” treatment would be similar to that of “broth” (Fig. 2B).

3. Non-native *Methylobacterium* are harmful to the host. In this case, we expected the fitness of “other” plants to be lower than that of “broth” and “own” plants (Fig. 2C).

4. *Methylobacterium* may confer a general fitness advantage that is not specific to their native host landrace. In this case, we expected the fitness of “own” and “other” treatments to be similar, and higher than “broth” (Fig. 2D).

To test these possible interactions, we measured multiple phenotypes relevant for vegetative growth as well as reproduction. We note that the broth control would have more available nutrients than the other treatments, where the bacteria would convert a substantial fraction of nutrients into biomass. Therefore, we would expect that the growth medium alone would have a greater ability to support plant fitness in the broth control. Hence, compared to the broth control, we would expect to obtain a conservative estimate of the impact of bacterial inoculation.

The impact of *Methylobacterium* strains varies across landraces

Two important crop plant traits are growth rate and seed yield, which serve as indicators of plant fitness. During the growing period, the total increase in plant height (i.e., T3 – T1, Fig. 1A) was broadly similar across all four treatments. However, treatment with broth alone or broth containing *Methylobacterium* reduced growth compared to untreated plants of Chakhao and Phou-ngang (Fig. 3A; S3A Table; S3 Fig). In Phou-ngang, treatment with non-native (“other”) *Methylobacterium* caused a further reduction in growth compared to the other two treatments (Fig. 3A).

In contrast, total grain yield (i.e., total grain weight per plant) was typically higher for treated plants of all landraces, with the highest yield observed in broth control and/or “own” treatments (Fig. 3B; S3A Table). These patterns were robust to the inclusion of potential outliers (Fig. 3). The overall weak impact of native *Methylobacterium* on vegetative growth was also observed for other growth-related traits (S4 Fig), while additional reproductive traits such as the number and proportion of filled grains increased in at least in two landraces, Chakhao and Phouren-mubi (S5 Fig). Thus, *Methylobacterium* tended to increase grain yield but reduced vegetative plant growth across landraces.

To better understand these effects and test our specific predictions (Fig. 2), we calculated the difference in median trait values for the following pairs of treatments, for each landrace. (a) Broth control – Untreated, which is a measure of the effect of broth alone (b) Own – Broth control, a measure of the effect of own *Methylobacterium* over and above any broth effects, and (c) Other – Broth control, reflecting the effect of non-native *Methylobacterium*. Surprisingly, broth alone had a significant impact on nearly all plant traits, with a substantial (2–4 fold) increase in the number and fraction of filled grains as well as total grain yield in broth-treated plants when compared to untreated plants of two out of three landraces (Fig. 4A–B; S3B Table). On the other hand, growth rate and plant height showed a significant though small reduction due to broth in two landraces. All other broth effects were observed in only a single landrace. Altogether, broth enhances some (but not all) aspects of plant fitness. Note that one limitation of our experiment is that we could not implement a randomized block design. However, it is unlikely that systematic environmental gradients explain our results, because we do not see that the “other”

or “broth control” treatments being consistently the best or the worst across landraces (Fig 1A).

Next, we tested the impact of the specific phyllosphere *Methylobacterium* associated with each rice landrace, after accounting for the broth effects described above. Since the “own” treatment included broth, any differences between the broth control and “own” can be attributed to the bacteria. As observed for the broth control, the magnitude of impact of “own” *Methylobacterium* also varied across landraces (Fig. 4C; S3C Table), though there were some qualitative similarities in that all significant impacts were positive (Fig. 4D). The number of tillers and % filled grains tended to be higher in all landraces, though this was significant only in one landrace. In Chakhao, *Methylobacterium* exaggerated the effect of broth on grain filling, with a 30% increase in number of filled grains and a 13% increase in grain filling. However, in Phouren-mubi, *Methylobacterium* only increased the number of tillers; and like in Chakhao, lead to a small increase in the flag leaf length. Finally, in Phou-ngang, we did not observe a significant change in any of the traits. Thus, in contrast to the broth treatment, own *Methylobacterium* had relatively rare, though in some cases very large impacts on their host plants. These results partially support our second prediction that *Methylobacterium* are beneficial for their host plants.

Finally, we found that treatment with “other” (non-native) *Methylobacterium* was consistently deleterious, with large reductions in grain filling and yield relative to the broth control (Fig. 4E–F; S3D Table). In many cases, the reduction in trait values in the “other” treatment was similar to or lower than the observed benefit of the broth. For instance, Chakhao plants in the broth control treatment had ~300 more filled grains per plant when compared to untreated plants (Fig. 4A). But plants in the

“other” treatment had ~300 fewer grains than broth control, suggesting that the “other” *Methylobacterium* prevents the plant from gaining the benefits of the broth. Note that the same strain was used for the “other” treatment of all landraces; hence, it is also possible that this strain is peculiar in its inability to provide fitness benefits, or imposes a general fitness cost on rice hosts. Further, since this bacterial strain was originally isolated from a rice landrace from Arunachal Pradesh (a different state in the region), the landraces used in our experiments may not have encountered this particular strain in the past, and therefore may not be able to derive fitness benefits from that strain.

Overall, in Chakhao, inoculation with landrace-associated phyllosphere *Methylobacterium* had a large positive impact on yield-related traits but little impact on vegetative growth. In contrast, in Phouren-mubi, the native *Methylobacterium* strain increased aspects of vegetative growth (tiller number) but tended to reduce grain filling compared to the broth control, though this was not significant. Interestingly, broth alone had a very large effect on grain filling in this landrace (~1000 more grains per plant compared to untreated), potentially masking any benefits that may be conferred by the bacteria. Finally, in the case of Phou-ngang, we did not find any significant host plant benefits from phyllosphere-associated *Methylobacterium*. Altogether, these results seem to support our third prediction that non-native *Methylobacterium* are detrimental (but see the discussion for caveats); but contradict our fourth hypothesis stating that the benefits of *Methylobacterium* are generalized. Thus, while rice plants do not acquire similar benefits from randomly chosen *Methylobacterium* strains, native bacterial strains are also not guaranteed to be beneficial across landraces.

The phyllosphere microbiome is unaltered by *Methylobacterium* inoculation

Several studies indicate that the beneficial effects of some bacteria may involve the exclusion of harmful colonizers, or facilitation of other growth-promoting bacteria. To explore the causes of the observed impact on host fitness and yield, we first tested whether the inoculated *Methylobacterium* strains successfully colonized the experimental plants. Unexpectedly, two months after the foliar spraying we only found a few *Methylobacterium* colonies in leaf imprints (S6 Fig). Most recovered strains (identified using 16S rRNA sequencing) did not match the experimentally inoculated strains; except in Chakhao, where the inoculated “own” strain appeared to have colonized the treated plants (S5 Table). Interestingly, the “other” *Methylobacterium* strain was not observed in any landrace, suggesting that this strain is a poor colonizer of these landraces in the environmental conditions prevalent in Manipur. These results also suggest that the effect seen on yield must have been due to the immediate interactions with the host after inoculation.

Next, we tested whether the impact of *Methylobacterium* inoculation on host plant fitness might be correlated with altered composition of the phyllosphere microbiome. We focused on Chakhao because it showed the strongest fitness benefits from its native *Methylobacterium*. By the time of harvesting, the flag leaf microbiome was not significantly different across treatments (Fig. 5A). Further, we tested whether specific functional groups of bacteria were enriched or depleted across treatments. We used broad taxonomic identification of community members to determine the relative abundance of all bacteria from the genus *Methylobacterium* (“total M”); taxa that were closely related to the experimentally inoculated “own”, “other” or environmental

Methylobacterium; as well as common pathogens of rice plants such as *Burkholderia*, *Pseudomonas*, and *Xanthomonas*. As expected, the genus *Methylobacterium* constituted ~10-20% of all bacterial reads of phyllosphere microbiomes in all treatments (Fig. 5B). Surprisingly, the proportion of pathogens was largest in untreated plants and lowest in the “other” treatment (Fig 5B), and was not correlated with the abundance of all *Methylobacterium* (Spearman’s $\rho = -0.13$, $p = 0.6$) or of the inoculated (own) *Methylobacterium* (Spearman’s $\rho = -0.144$, $p = 0.63$). Interestingly, the “own” *Methylobacterium* strain constituted 60-70% of all *Methylobacterium* across treatments (including plants inoculated with the “other” *Methylobacterium*; Fig. 5C), confirming that this specific strain successfully colonizes Chakhao plants in the field, as observed from leaf imprints. We also observed additional (likely environmentally acquired) *Methylobacterium* strains on all plants – though at a much lower abundance – and very rarely, we saw strains that closely matched the inoculated “other” strain (Fig. 5C). In the other two landraces, abundance of pathogens was generally lower than in Chakhao, and the inoculated native *Methylobacterium* strains were also not abundant (S7 Fig).

We note here some limitations of these data. First, the low sample sizes for some treatments limits our ability to draw strong conclusions about microbiome variation. Since we collected the flag leaf microbiomes during harvesting, we also do not know whether the observed bacterial taxa colonized the flag leaves during or after plant growth or reproduction, impeding our ability to infer causal links. Third, because the 16S rRNA gene offers limited resolution, we cannot be certain about accurate identification of the “own” and “other” *Methylobacterium* strains. Finally, we do not know the provenance of the *Methylobacterium* strains, since in field experiments it was not possible to prevent colonization by naturally-occurring strains in the soil or

air. Hence, we cannot distinguish between colonization by such environmental “own” *Methylobacterium* vs. potential contamination across treatments. Nonetheless, our results do not support the hypothesis that *Methylobacterium* inoculation influences host fitness by altering phyllosphere colonization by pathogens; or more broadly, by altering the phyllosphere microbiome. Given that the “own” *Methylobacterium* strain seemed to be abundant on plants in all treatments, we hypothesized that events soon after experimental inoculation by *Methylobacterium* may explain the observed benefits for the host. Hence, we tested whether *Methylobacterium* influences early expression of important host plant signalling pathways.

Early host plant gene expression following *Methylobacterium* inoculation

Next, we determined whether *Methylobacterium*-rice interactions may have altered the expression of host genes involved in growth and defence, contributing to the observed phenotypes. We conducted a laboratory and greenhouse experiment where we exposed Chakhao seeds to the same four treatments as the field experiment and analysed expression of selected host genes. We destructively sampled seedlings at different stages, planting them in the greenhouse to monitor the early growth phase. Supporting our field results, we did not find a growth advantage of *Methylobacterium* during the early vegetative growth phase (Fig. S8).

We analyzed early gene expression from stored samples collected before vs. after soaking seeds (before transplantation); and before vs. after the foliar spray (soon after transplantation). We used qRT-PCR to measure the expression of genes

known to play important roles in plant responses to pathogens, herbivores, and abiotic stresses (Fig. 6A), relative to a housekeeping gene. Most genes did not show a significant impact of treatment or a time x treatment interaction, when considering pairwise differences before vs. after inoculation (either for the first or the second bacterial inoculation; S6 Table). The only exceptions were *SNAC1* and *PR1#74*, which showed significant or nearly significant impacts of time, treatment, and their interaction (*SNAC1*, ANOVA: time, $P = 0.07$, Cohen's $f = 0.471$; treatment, $P = 0.05$, Cohen's $f = 0.76$; time x treatment, $P = 0.07$, Cohen's $f = 0.72$; *PR1#74*, ANOVA: time x treatment, $P = 0.07$, Cohen's $f = 0.73$). *SNAC1* is involved in drought resistance [32], and it was upregulated before spraying only in untreated plants (Fig. 6). This upregulation indicates that the plants experienced conditions similar to drought stress. Interestingly, the remaining treatments did not show a similar increase in *SNAC1* expression, hinting that the broth and *Methylobacterium* treatments might provide drought tolerance. *PR1#74* is usually upregulated under pathogen attack; in our case, it was only upregulated in the “other” *Methylobacterium* treated plants. Although this supported the idea plants respond to this particular *Methylobacterium* strain as if it were a pathogen, these results do not explain the positive impacts of the own *Methylobacterium* and of broth alone. Other genes involved in pathogen response – *OSXA21* and *FLS2* – were not upregulated, which is expected because they specifically respond to bacterial factor *Xoo* (*Xanthomonas oryzae*) and flagella, neither of which are present in *Methylobacterium*. Altogether, our gene expression analyses suggest that some host plant responses to environmental stresses may be altered by *Methylobacterium*. However, none of the observed effects (which are also weak) correlate with patterns of increased host fitness due to the own *Methylobacterium*, and therefore cannot explain the broad fitness effects. Hence, the

mechanistic basis of the fitness benefit of native *Methylobacterium* as well as the bacterial growth media remains unclear.

Auxin production by *Methylobacterium* strains

Finally, we tested whether differential auxin production by the different *Methylobacterium* strains used in our study could explain their variable fitness impacts. We compared our results to a reference strain of *Methylobacterium oryzae* (KACC 11585) that encodes some of the relevant metabolic pathways and hence serves as a positive control. Surprisingly, during growth in culture media with different carbon sources, all three native *Methylobacterium* strains produced a substantial amount of auxin when compared to the reference *Methylobacterium oryzae* (Fig. 7). However, the amount of auxin produced was not correlated with the relative fitness benefit conferred on the host plant. For instance, the highest amount of auxin was produced in the presence of methanol, by the strain associated with Phou-ngang (first two panels of Fig. 7); but this strain had no impact on host plant growth or yield. Hence, overall, in vitro assays did not support the hypothesis that landrace-specific impacts on host fitness could be explained by differences in the strains' ability to produce auxin that may influence plant growth.

Discussion

Our work represents one of the first field analyses of the impact of phyllosphere bacteria on their host plants. Previously, we showed that leaves of different rice landraces are colonized by phenotypically distinct strains of *Methylobacterium*, with

preliminary greenhouse experiments indicating landrace-specific benefits for plant growth [19]. These results suggested that host selection might have driven specific *Methylobacterium*–rice landrace associations in paddy fields; and that this could be utilized for increasing rice yields. Our results from the field experiment described here support the broad hypothesis that phyllosphere *Methylobacterium* benefits their host, and that the effect varies across landraces. In contrast to our earlier greenhouse experiments, the field study did not find a large impact of *Methylobacterium* on vegetative growth traits. The divergent outcomes could reflect the different timescales of the experiments (greenhouse experiments were terminated at 40 days); differences in the inoculation protocol (in the greenhouse experiment, seeds were sterilized before inoculation); or different environmental conditions, including the pool of available environmental microbes and multiple abiotic factors that cannot be controlled in the field. However, in the current study, we did find significant impacts of *Methylobacterium* on yield-related traits (~2 to 6 fold increase), including the number and fraction of filled grains. Remarkably, Chakhao plants inoculated with their native *Methylobacterium* strain produced ~1200 grains – substantially more than untreated plants (~600) or plants treated with the broth control (~900) or non-native *Methylobacterium* (~700). Thus, for highly valued landraces such as Chakhao and Phouren-mubi, inoculation with *Methylobacterium* could be developed as a method to improve yield.

Importantly, these benefits seem to depend on the specific association between each landrace and the predominant *Methylobacterium* strain that it hosts. A potential cause of such host-specific benefits is that host genotype determines interactions with bacterial partners, as observed in many other plants [44]. In our case, such

574 differences might mean that varieties such as Phou-ngang were unable to establish
575 a mutualism with *Methylobacterium* – e.g. due to differences in leaf architecture or
576 surface chemistry. For instance, colonization of different tomato cultivars by
577 *Salmonella enterica* varies depending on the presence of type-1 trichomes on
578 tomato leaves [45]. A second possibility is that properties of the different strains
579 might determine host fitness impacts. For instance, the Chakhao-associated strain
580 might be generally more beneficial, compared to the strains selected for Phouren-
581 mubi or Phou-ngang. However, our assay of auxin production did not support the
582 idea that the most beneficial “own” strains were distinguished by excess production
583 of plant growth promoting factors. We note that this analysis was limited to a single
584 metabolite measured *in vitro* rather than during plant colonization. The strains might
585 also differ in their ability to colonize different landraces under competition, as
586 observed for phyllosphere *Methylobacterium* of *Arabidopsis* [46]. In our case, strains
587 identical or similar to the “own” *Methylobacterium* dominated the phyllosphere
588 microbiome across all treatments. Prior work showed that only some
589 *Methylobacterium* strains successfully colonized rice and increased grain filling [16].
590 Mirroring our results, this study also found that some strains either had no impact on
591 or reduced rice growth or grain filling. Importantly, our non-native “other”
592 *Methylobacterium* strain appears to be harmful for all landraces, though the
593 mechanisms underlying this effect remains unclear. We also note that due to
594 logistical limitations we only included one “other” and “own” strain in our study, and in
595 future work it will be important to include more strains of each category to be able to
596 generalize the effect of native vs. non-native bacteria. We also note the possibility
597 that the “own” strains that we identified as native strains colonizing Phouren-mubi
598 and Phou-ngang [19] were perhaps only transiently dominant and are not

consistently associated with these landraces; or that their association is relatively recent. Lastly, variability in the impact of bacteria on host fitness may be driven by more complex interactions between multiple causal factors. For example, genetic differences across landraces may have led to divergent host-imposed selection on their bacterial associates, such that *Methylobacterium* associated with Phouren-mubi and Phou-ngang evolved to confer fitness benefits that would only be visible under specific environmental conditions. Indeed, leaf nutrients and soil condition together influence the phyllosphere microbial community across different genotypes of rice plants [47]. Similarly, the degree of prior adaptation to the local environment may also influence host-rice interactions. Phou-ngang is typically not cultivated in the region where our field site was located; perhaps it does benefit from its native *Methylobacterium* strains when cultivated in other areas. To distinguish between all these hypothesis, future work must analyse the temporal and spatial robustness of rice phyllosphere communities and conduct more extensive field trials to screen factorial combinations of rice landraces and *Methylobacterium* strains under diverse growing conditions. We hope that such analyses will improve our ability to predict which strains may or may not enhance host plant fitness.

Strikingly, in both Phouren-mubi and Chakhao, we found that *Methylobacterium* significantly reduced grain sterility (~20%) compared to control plants (~50%). What mechanisms might underlie these strong impacts? A recent meta-analysis shows that the effect size of beneficial microbes on plant health tends to be greater under stress [48]. In Manipur, 2018 was a drought year, with the worst water shortage occurring during the reproductive phase, causing a severe loss in yield across the region (personal communication with farmers). Our field experiment might have been

partially affected by the drought, although we tried to mitigate the effects (see Methods). Prior work shows that grain filling in rice is sensitive to drought, because drought induces excess ethylene production (a stress hormone) that regulates the grain filling rate [49–51]. Interestingly, bacteria such as *Pseudomonas putida* and *Pyrococcus horikoshii* can reduce stress-induced ethylene levels by secreting 1-aminocyclopropane-1-carboxylic acid deaminase, which breaks down the precursor of ethylene (reviewed in [52]. Similarly, *Methylobacterium fujisawaense* reduces ethylene levels in Canola, enhancing plant growth [53]; and *Methylobacterium* sp.2A isolated from the rhizosphere of potato plants improved plant growth under adverse conditions [54]. Thus, we speculate that in our experiment, the impact of *Methylobacterium* was at least partially mediated by the regulation of ethylene levels in response to drought stress. Future analyses are required to explicitly test this hypothesis.

A curious and unexpected result of our experiments is the strong beneficial impact of soaking seedlings and foliar spraying with bacterial growth media (“broth”). This effect could potentially be explained by methanol, which we added to the broth as a carbon source for bacterial growth. During plant growth, methanol is released as a by-product of cell-wall remodelling due to the activity of pectin methyl esterases (PMEs). The application of methanol on leaves leads to upregulation of PME expression, resulting in plant growth promotion, as observed in tobacco and *Arabidopsis* [55]. Another field experiment also indicated that foliar spray of 30% methanol enhances colonization and cytokinin production by *Methylobacterium*, increasing the growth of cotton and sugarcane [56]; (though this study had very small sample sizes). However, our growth media contained very little methanol

(~0.125%; S1 Table). Further, broth (and indeed all treatments that included the growth media) actually tended to reduce growth related traits in our experiments. Hence, it is unlikely that in rice, the impact of the broth is similar to the growth-promoting effects observed in previous studies. We did observe a very strong effect of broth on yield related traits in two landraces. What could explain this effect? One possibility – that the inoculation of broth enhances colonization by beneficial environmental microbes – is not strongly supported by our data, since we did not observe a significant difference in the phyllosphere microbiomes of untreated and broth-treated plants. However, it is possible that the microbiome changed at a critical early stage or in a different plant site such as the rhizosphere (which we did not sample). Alternatively, our low sample sizes may have limited our ability to determine differences in phyllosphere microbiomes. Finally, the broth includes trace metals that can alter host plant gene expression at low concentrations [57,58], potentially increasing grain yield. This is an intriguing possibility that should be explored further to understand the physiological mechanisms and potential agricultural applications. Finally, as mentioned in the results section, we likely underestimated the impact of bacteria on plant growth because of more nutrient availability in the broth control. Hence, we suggest that future work should use spent filtered growth media as a control.

In summary, our work highlights an important step in understanding and developing host-bacterial interactions for agriculture applications. Our results support a growing body of work showing that phyllosphere microbiomes are strongly shaped by local filtering imposed by host plants, and that the fitness effects of bacteria on their hosts are not generalizable. Thus, inoculating traditionally cultivated rice varieties with

specific *Methylobacterium* strains is a promising targeted method to mitigate the impact of environmental stress on grain filling and enhance productivity.

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Author contributions

PS conceived, designed and conducted experiments, analysed data and drafted the manuscript. PVS designed experiments and provided reagents and laboratory facilities. DA conceived and designed experiments, directed analysis, wrote the manuscript, and acquired funding.

Data availability

Raw sequence data will be deposited in the Sequence Read Archive (SRA) of National Center for Biotechnology Information (NCBI). All the data supporting the findings are given with the paper as supplementary file SI_Datasheet1.

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Figures:

Fig. 1: Experimental design. (A) Timeline of the field experiment with key events. (B) Schematic showing the field design. The total number of plants transplanted is given in parentheses. (C) A view of the field site one month after transplantation.

Fig. 2: Illustration of possible outcomes of the field experiment, and their implications. (A) *Methylobacterium* does not provide a host fitness advantage relative to the broth control. (B) *Methylobacterium* is only beneficial for its “own” landrace. (C) Non-native *Methylobacterium* are harmful to the host. (D) *Methylobacterium* confers a general fitness advantage that is not specific to the native host landrace.

Fig. 3: The impact of *Methylobacterium* inoculation varies across landraces.

Boxplots show (A) plant growth rate (change in height) and (B) yield, as a function of different treatments across landraces. Sample size (number of replicate plants) is indicated in parentheses; asterisks indicate significant pairwise differences ($p < 0.05$), when including all replicates (grey) and when excluding influential data points (black), as estimated using GLM/ANOVA followed by Tukey's HSD.

Fig. 4: Summary of the impact of experimental treatments on plant phenotype.

Bar plots show the impact of (A,B) broth alone (compared to untreated) (C,D) own *Methylobacterium* (relative to broth) (E,F) other *Methylobacterium* (relative to broth) on all plant traits measured in this study, after removing influential data points (SI Datasheet1). The bar height indicates the difference in median trait values across the two relevant treatments. Asterisks indicate values that are significantly different from zero ($p < 0.05$); p values close to 0.05 are noted explicitly (S4 Table). Panels B, D and F, show the number of landraces in which a significant effect was observed (positive or negative); e.g., “2” indicates that two landraces showed a similar impact of the experimental treatment.

Fig. 5: Phyllosphere bacterial communities as a function of experimental treatments for the landrace Chakhao. (A) Linear discriminant (LD) plot showing the clustering of phyllosphere bacterial communities across treatments. Number in parentheses indicate the number of biological replicates in each treatment. Axis labels indicate the proportion of variation explained, and ellipsoids represent 95% confidence intervals. (B–C) Boxplots show the relative abundance of (B) All *Methylobacterium* (total M) vs. own *Methylobacterium* vs. pathogens (see methods for details) and (C) Own, other, vs. environmental *Methylobacterium* strains. Chi-squared tests show the effect of treatment on the proportion of each group.

Fig. 6: Impact of *Methylobacterium* on early gene expression in host plants. (A) Schematic showing key signalling pathways and genes that regulate plant responses to abiotic and biotic stresses. (B–D) Change in the expression of each gene before and after two rounds of bacterial inoculation (initial vs. 48 hpi of seedlings; before vs. after foliar spraying on 45 day old plants), normalized to the expression of an internal control (*OsACTIN1*). Error bars represent the standard error from three biological replicates/treatment (three seedlings or plants).

Fig. 7: Auxin (IAA) production in *Methylobacterium* strains. Plots show the concentration of IAA produced by each *Methylobacterium* strain (isolated from Chakhao, Phouren-mubi, Phou-ngang, and non-native *Methylobacterium*; *M. oryzae* (KACC11585) used as a positive control; n = 3 biological replicates/strain) when grown on media supplemented with various carbon sources (25 mM methanol + 2 mM succinate, 25 mM methanol, or 2 mM succinate). Each point represents a replicate culture; horizontal pink lines indicate the median.

Supporting information

S1 Table: Nutrients received by each plant after foliar spraying of 10 ml broth (sterile media without bacteria).

Components	Composition	g/mL	g/plant
Phosphate solution (P)	K ₂ HPO ₄	0.00126	0.0126
	NaH ₂ PO ₄	0.001125	0.01125
Sulphate solution (S)	(NH ₄) ₂ SO ₄	0.00025	0.0025
	MgSO ₄ .7H ₂ O	0.0001	0.001
Metal Mix	ZnSO ₄ .7H ₂ O	0.00018	0.0018
	CaCl ₂ .2H ₂ O	0.00146	0.0146
	MnCl ₂ .2H ₂ O	0.000107	0.00107
	FeSO ₄ .7H ₂ O	0.0025	0.025
	(NH ₄) ₆ .Mo ₇ O ₂₄ .4H ₂ O	0.00018	0.0018
	CuSO ₄ .5H ₂ O	0.000187	0.00187
Methanol (60 mM)			0.6 mili moles
Succinate (2 mM)			0.002 mili moles

999 **S2 Table:** Primers used for qRT-PCR for rice gene.

Primer name	Sequence 5'-3'	Target gene	Ref
Xa21 F	CTCCCATTACTGCTCTTCGTCC	Xa21 receptor	Song et al 1995; Peng et al 2015
Xa21 R	GACGTGTTCCAAGATGCCAGCG		
OsFLS2 F	GTCGCAGCATTACACGAAGACG	Flagella receptor	Gomez et al 2000
OsFLS2 R	ACGCCGTTCTTGAAGTCCAGC		
OsPR1b F	CATGGTAGCCGCCATGGCAC	Pathogenesis-related gene	Mitsuhara et al 2008
OsPR1b R	GTTGCTGGAGTGGATCAGGCTG		
OsPR8 F	CATCGGCGCGCAGTTCACCGG		
OsPR8 R	GCGAGGATGACGCTGAGGTTG		
OsMPK3 F	CTCTCCCTCATCGCCTTGCTGTC	MAP kinases	This study
OsMPK3 R	CTGGTACTTGTTTCGTCACCTCG		
OsMPK6 F	GCATATGAATTGATGGATACTGATCTGC		
OsMPK6 R	CTGAGGTGGTACGAGCAAGTCC		
OsWRKY62 F	GCCGCTCTTCAGCCGATCG	WRKYs transcription factors	Cheng et al 2010; This study
OsWRKY62 R	GGTGGGCGTGGTGACCGGC		
WRKY13 F	TTTGGGAAAGCGTTGGATTAGT		
WRKY13 R	GCGCACACACACTCCAATC		
OsWRKY76 F	CCTCGACCTCTGCGTCGGG		
OsWRKY76 R	CTCATCTGAGTGACCTTCGC		
OsWRKY53 F	CTTCGTCACGGTCTCCTCGCTTC		
OsWRKY53 R	GCTGCGCGGACTTGAACCTG		

OsACO1 F	CATCAACATGGAGTTGCTCGC	ACC-oxidase	Yims et al
OsACO1 R	CGCACACGCTTGTAGTGGTC		2013
OsPAL1 F	CGGTCGTTCCCGCTCTAC		
OsPAL1 R	TCGCCGTTCCACTCCTT	Phenylalanine	Yims et al
OsPAL4 F	GGAGGACCAAGGAGGGT	ammonia-	2013;
		lyase gene	Solekh et
OsPAL4 R	GGAGTGTGTTGATGCGG		al 2019
Osg1 F	GTATGGGACAAAGGATCTCC	Beta-	Yims et al
Osg1 R	GTGATCCAGCCCTACTGCTG	glucanases	2013
OsPR1#74 F	GTATGCTATGCTACGTGTTTATGC		
OsPR1#74 R	GCAAATACGGCTGACAGTACAG		Mitsuhara
OsPR1#11 F	ACGCCTTCACGGTCCATAC	Pathogenesis-	et al
OsPR1#11 R	AAACAGAAAGAAACAGAGGGAGTAC	related gene	2008
		Transcription	Xiao et
SNAC1 F	GGGTGCTGTGTCGGCTGTA	factor	al 2013
SNAC1 R	CGACTGCGACGTAACCATGT		

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S3 Table: Pairwise comparisons across treatments, and treatment x landrace interaction effects on plant traits. Significant P-values are represented in bold font.

A. Treatment: Untreated vs. treated						
Model = ANOVA	Treatment		Landrace		Landrace x Treatment	
Plant traits	F	P	F	P	F	P
Growth rate	40.768	<0.001	202.685	<0.001	2.779	0.065
Height	33.344	<0.01	546.668	<0.001	4.193	0.0167
Flag leaf length	0.158	0.07	35.54	0.7	20.389	<0.001
Flag leaf width	28.843	<0.001	127.396	<0.001	3.587	0.03
Number of tillers	1.66	0.2	2.322	0.1	0.285	0.752
Number of panicles	12.6	<0.001	5.314	0.006	3.122	0.047
Yield	45.6	<0.001	13.42	<0.001	9.245	<0.001
Grain weight	1.06	0.3	2.43	0.09	0.415	0.7
Filled grains	13.46	<0.001	42.398	<0.001	4.733	<0.001
B. Treatment: Untreated vs. broth						
Growth-rate	16.62	<0.001	82.03	<0.001	0.72	0.5
Height	14.4	<0.001	229.1	<0.001	0.65	0.5
Flag leaf length	0.7	0.4	4.18	0.01	13.4	<0.001
Flag leaf width	16.14	<0.001	79.4	<0.001	3.08	0.05
Number of tillers	0.058	0.8	0.44	0.6	1.5	0.2
Number of panicles	21.37	<0.001	1.89	0.15	5.44	0.006
Yield	53.22	<0.001	3.45	0.037	11.03	<0.001
Grain weight	2.33	0.13	3.14	0.049	1.14	0.3
Filled grains	49.56	<0.001	4.14	0.019	5.36	0.006
C. Treatment: Broth vs. own						
Growth-rate	0.005	0.9	100.52	<0.001	0.077	0.9

Height	0.43	0.5	269.6	<0.001	0.668	0.5
Flag leaf length	9.14	<0.001	0.94	0.4	1.33	0.3
Flag leaf width	0.239	0.6	74.3	<0.001	2.13	0.12
Number of tillers	9.607	<0.001	1.6	0.2	1.7	0.2
Number of panicles	0.1	0.7	6.26	0.003	0.412	0.6
Yield	0.92	0.3	18	<0.001	0.99	0.4
Grain weight	0.3	0.6	1.77	0.2	0.46	0.6
Filled grains	0.52	0.5	10.7	<0.001	0.73	0.5

D. Treatment: Broth vs. other

Growth-rate	14.6	<0.001	131.88	<0.001	2.4	0.096
Height	16.1	<0.001	366.6	<0.001	5.39	<0.001
Flag leaf length	0.91	0.3	1.408	0.25	0.561	0.6
Flag leaf width	5.84	0.018	42.88	<0.001	0.71	0.50
Number of tillers	0.075	0.78	0.38	0.7	1.84	0.16
Number of panicles	20.5	<0.001	2.628	0.078	3.4	0.04
Yield	18.6	<0.001	10.35	<0.001	2.55	0.084
Grain weight	1.81	0.18	4.55	0.014	0.46	0.6
Filled grains	14.14	<0.001	6.4	<0.001	1.03	0.36

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1011 **S4 Table:** Pairwise comparisons across treatments on plant traits after removing the
 1012 influential data points using GLM followed by Tukey's HSD. Significant P-values are
 1013 represented in bold font.

Landrace	Plant traits	P values		
		Untreated- Broth only	Own-Broth only	Other- Broth only
Chakhao	Growth rate	<0.001	0.97	0.99
	Height	0.05	0.77	0.75
	Flag leaf length	<0.001	0.001	0.87
	Flag leaf width	<0.001	0.07	0.71
	Number of tillers	0.98	0.15	0.95
	Number of panicles	0.96	0.83	0.78
	Yield	0.52	0.35	0.53
	Weight per grain	0.12	0.29	1
	Number of filled grains	0.76	0.066	0.97
	Percent filled grains	0.007	0.003	0.77
Phouren-mubi	Growth rate	0.24	0.98	0.75
	Height	0.59	0.68	0.28
	Flag leaf length	0.73	0.001	0.68
	Flag leaf width	0.012	0.65	0.3
	Number of tillers	0.95	0.003	0.89
	Number of panicles	<0.001	0.98	<0.001
	Yield	<0.001	0.99	<0.001
	Weight per grain	<0.001	0.78	<0.001
	Number of filled grains	<0.001	0.32	<0.001
Phou-ngang	Percent filled grains	<0.001	0.36	0.68
	Growth rate	<0.001	0.99	<0.001

Height	<0.001	0.94	<0.001
Flag leaf length	<0.001	0.56	0.02
Flag leaf width	0.72	1	0.94
Number of tillers	0.98	0.5	0.93
Number of panicles	0.088	0.85	0.1
Yield	0.03	0.96	0.21
Weight per grain	0.69	0.75	0.73
Number of filled grains	<0.001	0.71	0.04
Percent filled grains	0.94	1	0.45

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1016 **S5 Table:** *Methylobacterium* isolates identified from leaf imprints, 2 months after foliar spray.

Landrace	Samples	Total		Number of	
		<i>Methylobacteriu</i> <i>m</i> isolates	Number own/other isolates	of environmental isolates	
Chakhao	Untreated	2	0	2	
	Broth				
	control	5	2 (own)	3	
	Own	10	4 (own)	6	
	Other	9	0	9	
Phouren-mubi	Untreated	6	0	6	
	Broth				
	control	3	1 (own)	2	
	Own	2	0	2	
	Other	1	1 (own)	0	
Phou-ngang	Untreated	7	1 (own/other)	6	
	Broth				
	control	1	0	1	
	Own	5	0	5	
	Other	0	0	0	

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1019 **S6 Table:** Model summary showing time and treatment effects on the expression of rice
1020 plant genes. Significant P-values are represented in bold font.

A. Time: Initial vs. 48 hpi						
Model = ANOVA	Time		Treatment		Time * Treatment	
Genes	F	P	F	P	F	P
XA21	1.08	0.3	0.05	0.98	0.05	0.98
FLS2	3.17	0.04	0.49	0.69	0.19	0.99
MAPK6	2.36	0.089	1.29	0.29	1.44	0.2
WRKY13	3.79	0.07	0.088	0.96	0.088	0.96
WRKY53	1.76	0.2	0.076	0.97	0.076	0.97
SNAC1	4.61	0.047	0.311	0.81	0.311	0.81
PR1#74	4.7	0.045	1.4	0.3	1.4	0.3
OsPAL4	2.34	0.145	0.73	0.54	0.73	0.54
OsACO1	4.92	0.04	1.12	0.37	1.12	0.37
OsG1	0.6	0.45	3.7	0.05	3.7	0.05
B. Time: Before vs. after spraying						
XA21	0.9	0.35	0.37	0.77	0.744	0.5
FLS2	1.57	0.23	0.66	0.6	0.35	0.8
MAPK6	1.27	0.27	1.71	0.2	2.22	0.12
WRKY13	0.688	0.419	0.39	0.76	0.97	0.43
WRKY53	2.463	0.13	0.21	0.89	0.95	0.44
SNAC1	3.546	0.07	3.08	0.06	2.75	0.077
PR1#74	0.92	0.35	0.69	0.56	2.83	0.07
OsPAL4	0.63	0.44	1.17	0.35	1.06	0.39
OsACO1	0.46	0.5	0.44	0.72	0.138	0.94
OsG1	1.85	0.19	0.47	0.7	1.2	0.34

S1 Fig: Experimental field plot. A. View of the experimental field plot before and after transplantation of the rice plants. B. Inoculum used for treating the seedlings and foliar spraying on the rice plants. C. Transplantation of rice seedlings with enough spacing using a wooden frame.

S2 Fig: Experimental design of greenhouse experiment. Schematic showing the timepoints for treatments and RNA extraction to analyze host gene expression after bacterial treatment.

S3 Fig: Change in plant height over time in field experiment. Change in average plant height ($\pm$ SD) across time (T1= 1 month, T2 = 1.5 month, T3 = 3 months after transplantation, Fig. 1) across treatments (n = 15 plants/treatment/landrace).

S4 Fig: Plant vegetative traits as a function of treatments in the field experiment. A. Flag leaf length; B. Total plant height (T3); C. Number of tillers; D. Number of panicles (n = 15 plants per landrace per treatment). Asterisks indicate significant pairwise differences ($p < 0.05$), when including all replicates (grey) and when excluding influential data points (black), as estimated using GLM/ANOVA followed by Tukey's HSD.

S5 Fig: Plant reproductive traits as a function of treatments in the field experiment. A. Yield (total grain weight); B. Average weight per grain; C. Number of filled grains; D. Percent filled grains (n = 12-15 plants per landrace per treatment). Asterisks indicate significant pairwise differences ($p < 0.05$), when including all replicates (grey) and when excluding influential data points (black), as estimated using GLM/ANOVA followed by Tukey's HSD.

S6 Fig: Colonization of plants by *Methylobacterium* in the field experiments. Pictures show plates with leaf imprints taken after two months of foliar spraying. *Methylobacterium* colonies typically appear pink after one week of incubation. Each plate was imprinted with three leaves (from 3 different plants per treatment).

S7 Fig: Phyllosphere microbiomes of experimental plants in the field. (A) Phouren-mubi (B) Phou-ngang. Linear discriminant (LD) plots show the clustering of phyllosphere bacterial communities across treatments. Numbers in parentheses indicate biological replicates in each treatment. Axis labels indicate the proportion of variation explained and ellipsoids represent 95% confidence intervals. Boxplot show the relative abundance of own, other, and environmental *Methylobacterium* strains, as well as pathogens (see methods for details). Chi-square tests show the effect of treatment on the relative abundance of each group.

S8 Fig: Plant growth in the greenhouse experiment. Plant height measured after 28th dpi (days post inoculation) in Chakhao plants (n = 10 plants/treatment). Pairwise comparisons across treatments were done using Tukey's HSD.