

Cooperative siderophore use stabilizes a protective leaf microbiome

Paolo Stincone ^{*1,2,3}, Lukas M. Braun¹, Caner Bağcı⁴, Marcelo Navarro-Diaz², Alicia I. Pérez-Lorente^{5,6}, Shane P. Farrell⁷, Daniel Gómez-Pérez⁸, Johanna Bode¹, Karoline Steuer-Lodd¹², Maryam Mahmoudi^{1,9}, Vasvi Chaudhry¹, Diego Romero^{5,6}, Allegra T. Aron¹⁰, Nadine Ziemert^{4,11}, Carlos Molina-Santiago^{5,6}, Eric M. Kemen^{*1,2,3}, and Daniel Petras^{*2,12}

- 1) University of Tuebingen, Center for Plant Molecular Biology, Tuebingen, Germany
- 2) University of Tuebingen, CMFI Cluster of Excellence, Interfaculty Institute of Microbiology and Infection Medicine, Tuebingen, Germany
- 3) Cluster of Excellence GreenRobust, Eberhard Karls University, Tübingen 72076, Germany
- 4) Translational Genome Mining for Natural Products, Interfaculty Institute of Microbiology and Infection Medicine Tübingen (IMIT), Interfaculty Institute for Biomedical Informatics (IBMI), University of Tübingen, Tübingen, Germany
- 5) Instituto de Hortofruticultura Subtropical y Mediterránea La Mayora, Universidad de Málaga-Consejo Superior de Investigaciones Científicas, Departamento de Microbiología, Universidad de Málaga, Málaga, Spain
- 6) Departamento de Microbiología, Universidad de Málaga, Bulevar Louis Pasteur 31 (Campus Universitario de Teatinos), Málaga 29071, Spain
- 7) University of Maine, Walpole, ME, USA.
- 8) Organisms and Ecosystems, Earlham Institute, Norwich, UK
- 9) Department of Molecular Biology, Max Planck Institute for Biology Tübingen, 72076 Tübingen, Germany
- 10) Department of Chemistry and Biochemistry, University of Denver, Denver, CO, USA.
- 11) German Center for Infection Research (DZIF), Partner Site Tübingen, Auf der Morgenstelle 24, 72076 Tübingen, Germany
- 12) Department of Biochemistry, University of California Riverside, Riverside, CA, USA

Abstract

Plant-associated microbial communities provide crucial protection against pathogens. Specialized metabolites play key roles in plant-microbe and microbe-microbe interactions and, ultimately, in plant health; however, the molecular mechanisms underlying their plant-protecting properties remain largely unknown. Nutrient deficiency (e.g., iron) on leaf surfaces creates intense competition among microbes, driving both antagonism and cooperation. Using a gnotobiotic *Arabidopsis thaliana* model and a synthetic leaf microbial community, we show that community stability and plant protection depend on cooperative siderophore exchange between the basidiomycete yeast *Rhodotorula kratochvilovae* and commensal *Pseudomonas* species. Removal of *Pseudomonas* caused a strong shift in the community metabolome and accumulation of the yeast siderophore rhodotorulic acid (RA). RA selectively promoted the growth of commensal *Pseudomonas* via TonB-dependent transporters, which are absent in pathogenic *Pseudomonas* strains. Inactivation of these transporter genes abolished RA

uptake, destabilized the synthetic community, and eliminated protection against *Pseudomonas syringae* infection. RA and *Rhodotorula* also induced host iron-deficiency and jasmonate-related defense metabolites, linking microbial cooperation to plant stress responses. These findings reveal that microbial siderophore exchange acts as a key mechanism that maintains stability in the phyllosphere microbiome. Rather than solely promoting competition, iron-binding compounds can serve as cooperative currencies that align microbial fitness with host protection.

Keywords: Bacteria-Yeast interaction, Iron, TonB-dependent transporters, Specialized metabolites, Plant protection

Main

Plant microbiomes are fundamental to the health and functioning of terrestrial ecosystems globally, regulating nutrient cycling, bolstering plant resilience, structuring biological communities, and suppressing pathogens, thereby enhancing ecosystem stability^{1,2}. In applied contexts such as habitat restoration, climate adaptation, and agriculture, the integrity of plant–microbiome interactions is critical for maintaining ecosystem services that humans depend on, including soil fertility, water retention, carbon sequestration, and sustainable food production^{3,4}. Given the importance of plant-associated microbiomes to both ecological functioning and human well-being, unravelling their dynamics is essential for predicting ecosystem responses to environmental change and for informing conservation, management, and land-use strategies.

Yet, plant-associated microbiomes worldwide are threatened⁵ by numerous stressors, including habitat degradation, pollution, and extreme climate^{6,7}, which shift the balance from mutualistic microbes to opportunistic pathogens⁸. Understanding how these complex microbial communities maintain stability and protect their host under stress is becoming increasingly urgent. The phyllosphere, the aerial parts of plants, represents one of the largest microbial habitats on Earth⁹, yet the mechanisms governing host-microbe and microbe-microbe interactions are poorly understood. Emerging evidence indicates that certain phyllosphere residents, including commensal *Pseudomonas* species, can suppress plant pathogens^{9,10} however, the specific molecular and ecological mechanisms mediating this protection remain unclear.

One likely driver is microbial competition for scarce resources such as iron, a micronutrient in minimal supply on leaf surfaces¹¹. To survive in iron-depleted environments, many microbes secrete siderophores, small, high-affinity iron-chelating compounds¹², that not only promote individual fitness but also mediate competitive and cooperative interactions

among community members. These molecules can strongly influence microbial assembly and function¹³.

Here, we investigated how siderophore-producing commensal microbes and siderophore uptake by the commensal *Pseudomonas* keystone species contribute to the protection of *Arabidopsis thaliana*. Prior work investigating the microbiome of natural *Arabidopsis thaliana* populations¹⁴ identified a known yet understudied yeast of the genus *Rhodotorula* as a keystone taxon within the phyllosphere, revealing predominantly positive associations with other microbial members, particularly commensal *Pseudomonas* strains (Fig. 1a-b). Using a sterile *Arabidopsis thaliana* model and a representative Synthetic Community (SynCom) composed of core phyllosphere microbial members, we combined dropout co-culture experiments, functional metabolomics, amplicon sequencing, pangenome analyses, gene knockouts, and plant phenotyping to dissect the mechanisms underlying community-level iron acquisition. Our data revealed how siderophore sharing and the presence or absence of TonB-dependent transporters shape microbial competitive dynamics, community stability, and plant protection. Our results provide new insights into the molecular and ecological processes that stabilize beneficial plant-microbiome associations and may have broader implications for microbiome stability in environmental and other host-associated systems, including those relevant to human health.

Results

***Rhodotorula* promotes the growth of commensal *Pseudomonas* and contributes to plant protection**

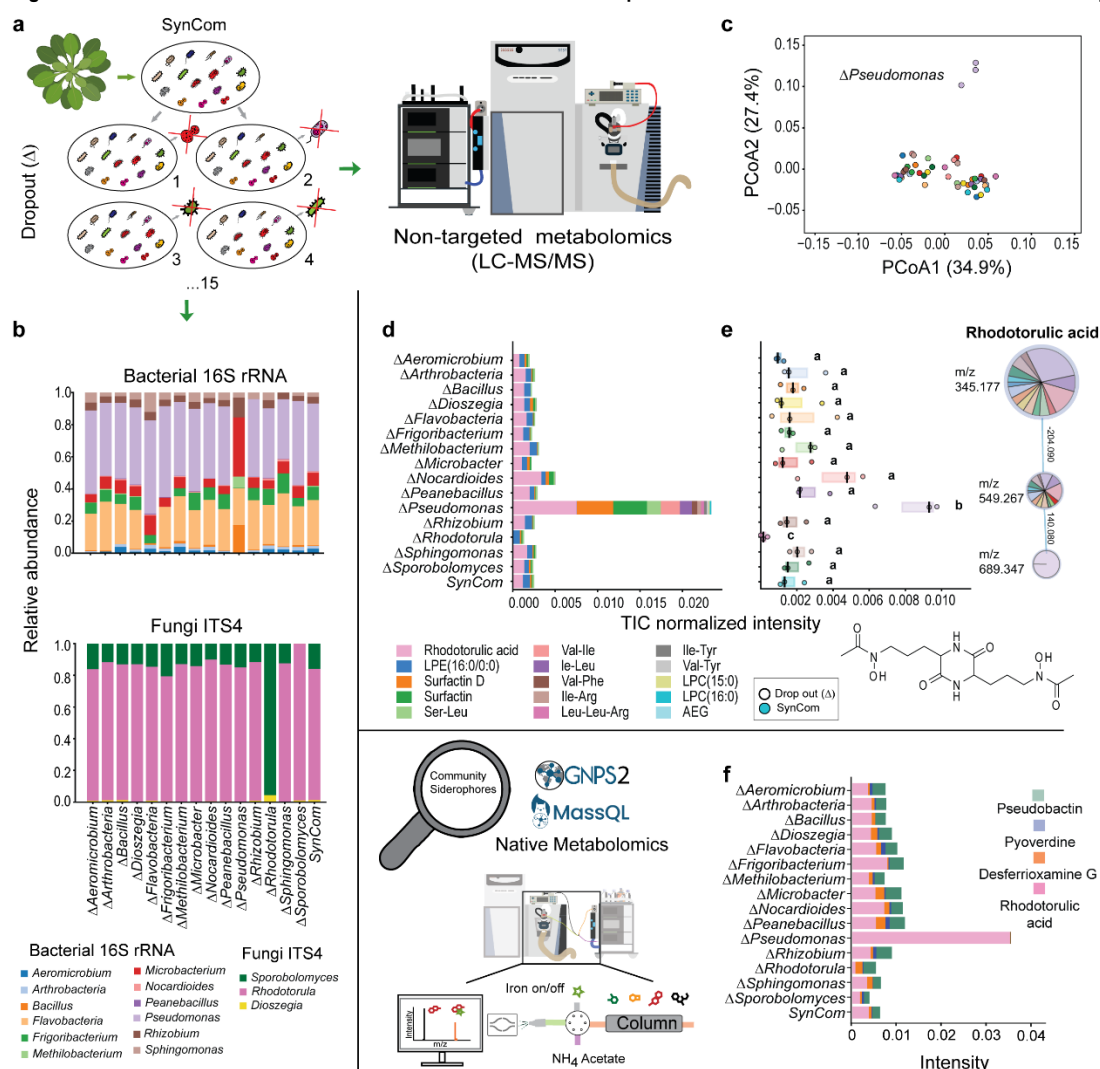
Field surveys conducted across six locations around Tübingen, Germany, over five years revealed strong positive correlations between *Rhodotorula* Operational Taxonomic Units (OTUs) and multiple bacterial taxa, including members of the *Pseudomonas* clade (Fig. 1a-b). These patterns suggested possible inter-kingdom facilitation or metabolic complementarity rather than antagonism within the microbiome. To validate these ecological associations experimentally, we used a previously established 15-member SynCom containing representative bacterial and yeast strains from the natural phyllosphere¹⁵. We quantified *in vitro* growth responses of all 15 SynCom members when exposed to the cell-free filtrate from *Rhodotorula kratochvilovae* cultures. Growth responses varied among SynCom members, ranging from stimulation to inhibition or no measurable change, confirming strain-specific sensitivity to *Rhodotorula* exometabolites.

***Pseudomonas* removal rewires the community metabolome and triggers rhodotorulic acid accumulation**

To quantify the contribution of individual community members to the core phyllosphere microbiome of *Arabidopsis thaliana*, we generated a series of dropout SynComs, each lacking one strain (Δ) from the initial 15-member consortium, which was used as a control (**Fig. 2a**). Amplicon sequencing and non-targeted metabolomics via liquid chromatography tandem mass spectrometry (LC–MS/MS) were used to profile both community composition and metabolic output. When *Pseudomonas koreensis* was omitted ($\Delta Pseudomonas$), bacterial genera such as *Bacillus*, *Microbacterium*, and *Methylobacterium* expanded to fill the vacated niche, whereas *Flavobacterium* and *Pseudomonas* remained the dominant taxa in complete SynCom cultures (**Fig. 2b**). *Rhodotorula* abundance, assessed by ITS4 reads, was not significantly altered by the absence of *Pseudomonas* and remained the most prevalent yeast across all dropout combinations. These data indicate that removing *Pseudomonas* primarily affects bacterial rather than yeast composition.

Across all co-cultures, 5663 LC-MS/MS features were detected after preprocessing, of which 763 differed significantly among dropout combinations (ANOVA, Benjamini/Hochberg False Discovery Rate (FDR) corrected $P < 0.05$; (**data S1**). Principal coordinate analysis (PCoA) of dropout SynCom metabolomic profiles revealed distinct separation, with $\Delta Pseudomonas$ clearly clustering from the other groups (**Fig. 2c**). Mean total ion chromatogram (TIC) intensities of the top 15 annotated metabolites with the highest ANOVA F-statistics indicate that RA is the most strongly differentiating metabolite among groups (**Fig. 2d**). RA abundance increased markedly in $\Delta Pseudomonas$ but was undetectable in $\Delta Rhodotorula$ cultures ($P = 3.37 \times 10^{-7}$) (**Fig. 2e**), consistent with the fungal origin of this siderophore and the capacity of gram-negative bacteria such as *Pseudomonas* to scavenge hydroxamate ligands of fungal origin.

Fig. 2 Molecular mechanisms behind the *Rhodotorula*-*Pseudomonas* positive correlation in the “core” microbial community.



a, Schematic representation of the dropout SynCom co-cultures assembled to investigate the chemical space occupied by each strain within the “core” microbiome. **b**, Relative abundance of strains in dropout SynCom co-cultures, assessed by 16S rRNA/ITS4 Nanopore amplicon sequencing from MM9 agar after 5 days of incubation at 22 °C. **c**, PCoA plot of dropout SynCom metabolomic profiles, samples colored by dropout. **d**, Absolute stacked bars show mean TIC values of the 15 annotated metabolites with the highest ANOVA F-statistics across groups. **e**, Boxplot of TIC-normalized intensity of the annotated node corresponding to rhodotorulic acid, identified from non-targeted metabolomics combined with Feature-Based Molecular Networking (FBMN). Different letters denote significant differences (ANOVA with Tukey’s test, $P < 0.05$). In this network, nodes represent detected features connected by MS² spectral similarity (cosine > 0.75), and edges indicate the corresponding delta-mass relationships. Node size reflects differences in feature intensity across groups. **f**, To focus on metabolites with iron-binding capacity within the community, GNPS molecular networking, MassQL queries, and metal infusion–based validation were applied to identify siderophores and assess their distribution across dropout SynCom extracts. Stacked bar plots display the relative abundance of the main siderophores identified by MassQL and/or metal-infusion native metabolomics for each dropout SynCom co-culture. Peak areas corresponding to nodes within the same cluster and with similar retention times were merged to obtain the most reliable estimates of the different siderophore concentrations among the group.

Because RA was identified as the metabolite most responsive to *Pseudomonas* removal, we examined siderophore production patterns in greater depth. In addition to compounds annotated via GNPS, we employed MassQL queries and metal-binding native metabolomics to uncover additional iron-chelating molecules (**Extended Data Fig. 2, and Supplementary Table S1**). RA was not detected by MassQL, likely due to its trimeric Fe₂RA₃ complexation, but was readily confirmed by the metal-infusion approach. When members

encoding siderophore biosynthetic gene clusters (BGCs; identified by antiSMASH) were removed, the diversity of detected iron-binding compounds decreased, consistent with intensified iron competition driven by siderophore-producing taxa. Conversely, excluding strains lacking siderophore BGCs increased siderophore diversity, suggesting that, when present, these taxa constrain siderophore producers through niche occupation and competition for iron, thereby limiting the diversification of siderophore profiles (**Extended Data Fig.2**).

Raw peak-area analysis across all combinations revealed that *Pseudomonas koreensis* produces pseudobactin and an uncharacterized low-weight pyoverdine siderophore, which stimulated other community members to secrete desferrioxamine G and RA (**Fig. 2f**). In the $\Delta Pseudomonas$ SynCom, RA dominates siderophore production, whereas *Pseudomonas* presence promotes diversification through iron competition. Taken together, these data demonstrate that removing *Pseudomonas* disrupts the community's metabolic equilibrium, resulting in compensatory production of the fungal siderophore RA. This cross-kingdom metabolic response suggests that *Pseudomonas* not only modulates iron competition but can also exploit siderophores secreted by other SynCom members, providing a mechanistic foundation for cooperative iron acquisition and microbiome stability.

Rhodotorulic acid selectively stimulates commensal but not pathogenic *Pseudomonas*

Our previous data indicate that the cell-free supernatant from *Rhodotorula kratochvilovae* cultures supports the growth of *Pseudomonas* in isolation (**Extended Data Fig.1**). We first confirmed that the supernatant used in this experiment is enriched in RA (**Extended Data Fig. 3a**). We then tested whether RA can support the growth of the SynCom member *Pseudomonas koreensis*. Supplementation of iron-limited MM9 medium with increasing concentrations of RA significantly stimulated the growth of the *P. koreensis* strain in a dose-dependent manner over 8–9 hours of incubation (**Extended Data Figs. 3A-B**). We also performed growth assays in MM9 supplemented or not with RA in the presence or absence of either glucose or amino acids (aa) to rule out the possibility that *P. koreensis* uses RA as a carbon or nitrogen source (**Extended Data Fig.4**). Altogether, our data indicate that RA enhances bacterial proliferation under iron-limited conditions by providing chelated Fe³⁺. Carbon limitation increases growth rate but simultaneously increases iron demand, making siderophore uptake a limiting factor.

To test the specificity of this interaction, we extended the analysis to additional *Pseudomonas* isolates representing both commensal and opportunistic pathogenic lifestyles. Growth stimulation by *Rhodotorula*-conditioned medium was consistently observed among commensal strains, whereas none of the pathogenic isolates, including those frequently associated with the *Arabidopsis* phyllosphere, showed a significant response compared with untreated controls (**Fig. 3a**). These results demonstrate that RA acts as a selective

xenosiderophore, accessible to commensal *Pseudomonas* but unavailable to pathogenic counterparts. By exploiting fungal siderophores, commensal *Pseudomonas* spp. gain an iron-dependent fitness advantage that may restrict opportunistic pathogens.

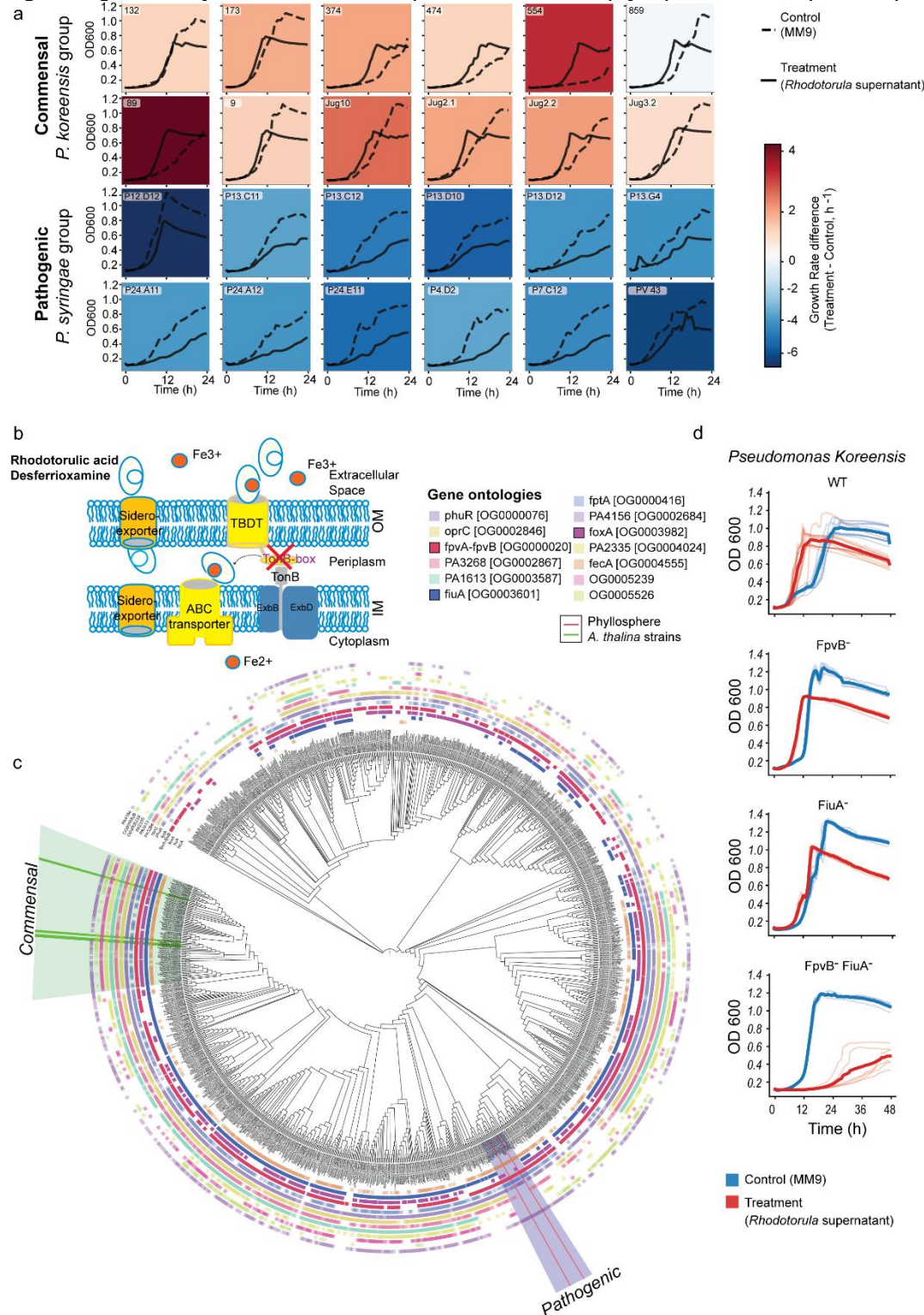
TonB-dependent transporters mediate RA uptake in commensal *Pseudomonas*

To uncover the genetic basis of RA utilization, we compared the genomes of commensal and pathogenic *Pseudomonas* isolates. Whole-genome sequencing and pangenomic analyses revealed pronounced differences in TonB-dependent transporter (TBDT) repertoires between lifestyle groups (**Extended Data Fig. 5a**). Genes encoding outer-membrane TBDTs with TonB-box signal sequences, which are essential for siderophore translocation, were consistently enriched in commensal strains but absent from pathogenic ones (**Fig. 3b**). These included gene clusters homologous to *fiuA*, which mediate the uptake of hydroxamate siderophores such as RA, as well as *foxA* involved in the transport of desferrioxamine (**Extended Data Fig. 5B**).

To determine whether these patterns extend across the genus *Pseudomonas*, we expanded our analysis to include all available species, revealing a consistent absence of these TBDTs within the *P. syringae* clade (**Fig. 3c**). Focusing further on dominant *Pseudomonas* species associated with wild Arabidopsis, we screened ~1400 publicly available *Pseudomonas viridiflava* genomes for Pfam-annotated domains required for functional siderophore uptake. This analysis showed that the genes encoding the TonB-box-containing transporters *fiuA* and *foxA* were consistently absent from pathogenic *P. viridiflava* genomes, whereas all three TBDTs were retained in leaf-isolated commensal strains (**Extended Data Fig. 6**).

To assess the role of TBDTs in siderophore uptake, we generated knockout derivatives of the SynCom commensal *P. koreensis* and monitored their growth in the presence of *Rhodotorula* supernatant¹⁶. Deletion of *fpvB*⁻, also involved in RA uptake, had no significant effect, whereas deletion of *fiuA*⁻ reduced growth promotion. The double mutant lacking both *fiuA*⁻ and *fpvB*⁻ (*fiuA*⁻*fpvB*⁻) failed to benefit from *Rhodotorula* supernatant, confirming that both receptors are essential to sustain the growth advantage observed in the wild type (**Fig. 3d**). The growth rate *fiuA*⁻*fpvB*⁻ was comparable to that of the pathogenic *P. viridiflava* strains (**Fig. 3a**). Importantly, iron supplementation rescued the growth defect of the double mutant in the *Rhodotorula* supernatant (**Extended Data Fig. 7**). These data suggest iron competition and capture is mediated through TBDTs, rather than carbon availability, as the mechanism underlying the differential responses of commensal and pathogenic *Pseudomonas* strains to *Rhodotorula* conditioned medium.

Fig. 3. Pangenome analysis reveals absence of specific TonB boxes for phylosphere core siderophores in pathogenic strains.



a, Growth curves of 12 commensal and 12 pathogenic *Pseudomonas* strains used in this study. All strains were grown in biological replicates in MM9 medium (dashed lines; control) or MM9 supplemented with 50% (v/v) *Rhodotorula*-conditioned medium (solid lines; treatment). The background heatmap indicates the difference in growth rate between control and treatment conditions, with the color gradient reflecting the magnitude of the effect. **b**, Schematic representation of siderophore transport in Gram-negative bacteria, highlighting the TonB box, which is essential for siderophore translocation into the periplasmic space. **c**, Distribution of TonB-dependent transporters (TBDTs) across the *Pseudomonas* genus, shown in the context of phylogenetic relationships. Annotated TBDTs and those differentially present between commensal (*P. fluorescens* complex) and pathogenic (*P. syringae* group) genomes are indicated. **d**, Deletion of TBDTs in a commensal *Pseudomonas* SynCom member abolishes the growth-promoting activity of the *Rhodotorula* supernatant.

Rhodotorulic Acid indirectly shapes the microbiome through plant iron stress responses

Because RA accumulated when commensal *Pseudomonas* were absent or lacked the corresponding transport system, we next examined how *Pseudomonas*, *Rhodotorula*, the siderophore RA, and their combinations influence the metabolome of sterile plants following infection by the pathogen *P. syringae* (**Extended Data Fig. 8A**). We speculated that interactions between *Rhodotorula* and *Pseudomonas* may confer indirect host protection by creating an iron-limiting environment that excludes pathogenic microbes. Non-targeted metabolomics of plant aerial tissue revealed that concomitant exposure to *Pseudomonas* and *Rhodotorula* or to *Pseudomonas* and RA interactions was associated with depletion of jasmonic acid (JA) precursors (**Extended Data Fig. 8**) relative to control and *Pseudomonas* only treatments. JA plays a central role in plant defense and is tightly linked to lignin biosynthesis, a pathway critical for cell-wall reinforcement and stress tolerance ¹⁷. Consistently, multiple lignin precursors were upregulated ¹⁸, suggesting a coordinated host response to iron deficiency that enhances structural defenses while facilitating iron mobilization ¹⁹. Among these compounds, sinapic acid, 3,5-dimethoxy-4-hydroxycinnamic acid, and sinapoylmalate were significantly upregulated under both RA and *Rhodotorula* treatments (**Extended Data Figs. 8a and 9**). Notably, sinapic acid was further detected to be metabolized by several SynCom core microbiome members, including *Pseudomonas*, *Sphingomonas*, *Nocardiodes*, *Dioszegia*, and *Bacillus*, suggesting a potential metabolic link between host defense metabolites and microbial community dynamics (**Extended Data Fig. 8b**).

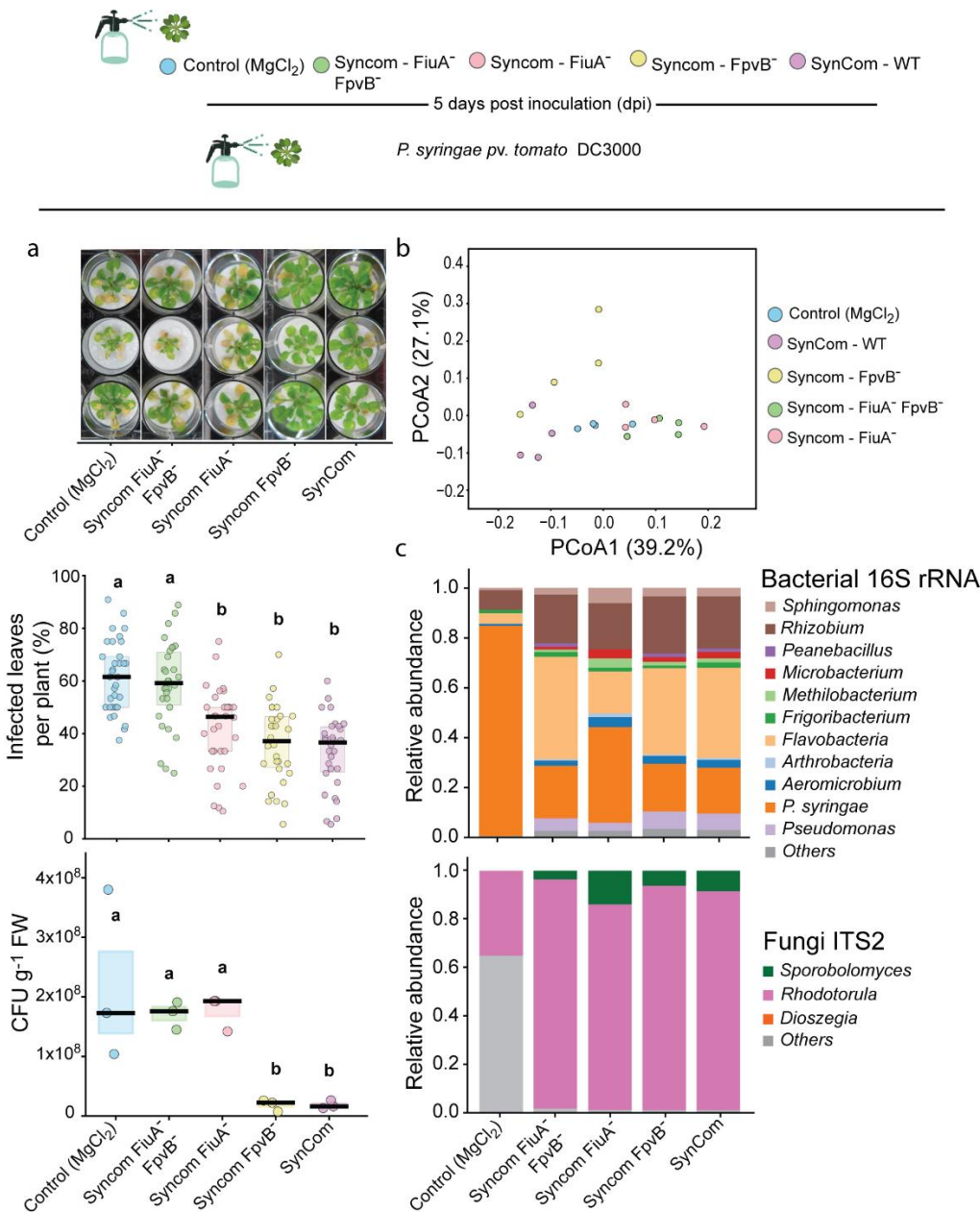
Role of *FiuA* and *FpvB* in plant protection against *Pseudomonas syringae*

To investigate whether TBDTs in *P. koreensis* underlie *Rhodotorula*-mediated plant protection in the context of the SynCom, we generated community derivatives in which the wild-type commensal *Pseudomonas* was replaced by each of the TBDT knockout mutants. These modified SynComs were inoculated onto gnotobiotic *A. thaliana* to assess loss or reduction of the protective phenotype compared to wild type (WT) SynCom. Following a 5-day establishment period, plants were infected with *P. syringae*, disease progression was quantified using both the percentage of symptomatic leaves per plant and Colony-forming units (CFUs) per gram of fresh plant weight (CFU g⁻¹ FW). A clear gradient in protection emerged: plants inoculated with the *fpvB* knockout SynCom exhibited disease levels comparable to the wild type community, whereas, the SynCom harboring *Pseudomonas fiuA*-

fpvB- showed the highest infection levels, indistinguishable from the non-inoculated control (Fig. 4a).

Non-targeted metabolomics of plant aerial tissues corroborated these patterns. Principal coordinates analysis (PCoA) and hierarchical clustering revealed that plants colonized with the *fpvB* knockout SynCom displayed metabolomic profiles similar to those receiving the wild-type community, while plants inoculated with *fiuA* and the double mutant SynCom clustered with the control samples (Fig. 4a and fig. S10). Our results show that polyunsaturated fatty acids and oxylipids (peroxidized fatty acids), direct precursors of jasmonic acid, are differentially regulated across treatments, in line with previous reports implicating jasmonic acid in iron-homeostasis responses (Extended Data Figs. 8a-b). Lignin precursors levels increased under conditions where iron availability may have been limited, potentially as a consequence of increased microbial competition for iron. Amplicon sequencing of the epiphytic microbial community confirmed that all *Pseudomonas* mutant strains persisted stably within the SynCom following plant inoculation (Fig. 4c). In control plants, which had not been exposed to any microbial community, a small number of reads were detected, likely reflecting minor cross-contamination. Together, these results demonstrate that TBDTs link microbial iron acquisition to host metabolic response, thereby shaping community-level protection against *P. syringae*.

Fig. 4. Interkingdom interactions promote plant protection.



A. thaliana plants were grown sterile condition and treated with 10 mM MgCl₂ containing either the complete SynCom or derivative communities in which *P. koreensis* was replaced by knockout strains generated in this study. Five days later, all plants, including controls, were inoculated with *P. syringae* at equal optical density. **a**, Disease severity, quantified as percentage of infected tissue and pathogen load (CFU g⁻¹ fresh weight). Different letters denote significant differences (ANOVA with Tukey's test, $P < 0.05$). **b**, Principal coordinates analysis (PCoA) of non-targeted metabolomics data, based on Bray–Curtis distances of TIC-normalized features. Points represent individual samples; colors indicate treatments. Metabolite profiles differed significantly among SynComs (PERMANOVA, pseudo- $F = 0.01$, $P = 0.026$). **c**, Relative abundances of SynCom members and *P. syringae* from epiphytic microbial DNA, including bacterial and fungal fractions.

Conclusion

Iron is a limiting micronutrient, and its reduced availability restricts pathogen colonization in many microbiomes, including the phyllosphere. Keystone microbial species, such as commensal *Pseudomonas* members, stabilize the phyllosphere. Disruption of plant-associated microbiomes compromises plant productivity, ecological stability, and stress resistance, underscoring the importance of maintaining microbiome integrity. Yet, the mechanisms underlying the protective effects of *Pseudomonas* mediated by microbial interactions remain largely unresolved. Using a synthetic community derived from natural *A. thaliana* microbiota and using state-of-the-art analytical tools, we show that *Rhodotorula* enhances the growth of commensal *Pseudomonas* through the uptake and utilization of RA, a process dependent on the exclusive presence of a TonB-dependent transporter (TBDT). Together with other community siderophores, such as desferrioxamine G, this interaction reduces iron availability in the environment, thereby shaping microbial interactions and influencing host-microbiome dynamics. In contrast, members of the pathogenic *P. syringae* group are unable to utilize xenosiderophores in the community, as confirmed by pangenome and *in-vitro* analysis (**Fig. 3b**). Commensal *Pseudomonas* produce their own siderophores, thereby intensifying competition for iron and limiting its availability within the plant microbiome. This severe iron scarcity reduces the survival capacity of pathogenic microbes, directly enhancing host protection. Furthermore, we found that spraying apo-rhodotorulic acid (apo-RA) triggers a host stress response, leading to the activation of the lignin biosynthesis pathway, which our results suggest is beneficially exploited by members of the core microbiome (**Extended Data Fig. 8b**). As such, our study identifies iron-mediated microbial competition as a key process in protecting and stabilizing plant microbiomes (**Fig. 5**).

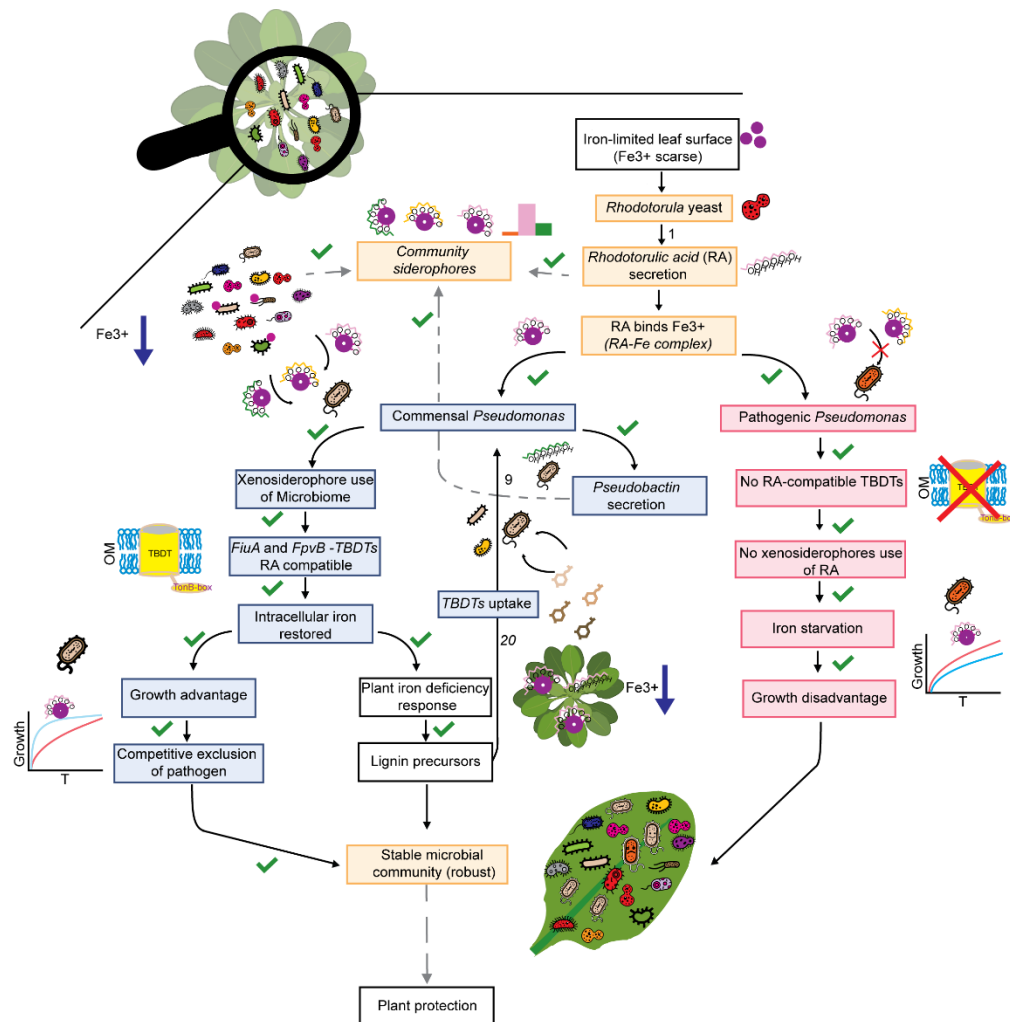


Fig. 5: Siderophore-mediated cooperation in phyllosphere iron acquisition promotes plant protection. Flowchart depicting the major mechanisms identified in this study.^{9,20}

Our findings extend the current understanding of interkingdom interactions in plant-associated microbiomes by demonstrating their role in enhancing host protection within the phyllosphere. While previous studies have documented beneficial interkingdom interactions in the rhizosphere²¹, similar dynamics in the phyllosphere remain underexplored. This is particularly relevant given the established role of the phyllosphere as a critical front line in plant defence against foliar pathogens²². Commensal *Pseudomonas* strains, such as those included in our synthetic community, suppress the growth of opportunistic *Pseudomonas* pathogens that are widely distributed across agricultural and wild *A. thaliana* populations¹⁰. Members of the genus *Pseudomonas* can act as both beneficial and pathogenic members of the phyllosphere microbiota²³. Iron acquisition has emerged as a key mechanism by which commensal *Pseudomonas* limit pathogen proliferation¹¹. In our system, the presence of yeast-derived siderophores, specifically RA produced by *Rhodotorula*, promotes directly commensal *Pseudomonas* growth while excluding pathogenic strains. We observed that the simple siderophore triggers plant immunity and lignin biosynthesis, with a beneficial effect on

members of the core microbiome, thereby indirectly stabilizing the community. These selective effects may facilitate niche dominance, reducing pathogen access to essential resources^{24–26}. Pathogen success depends not only on host susceptibility and nutrient availability, but also on the capacity to compete within a complex microbiome^{27–29}.

Our work shows that interkingdom cooperation in iron utilization based on the presence of TBDTs in keystone members, such as commensal *Pseudomonas* in the phyllosphere, shapes both plant health and microbial community structure, revealing an underappreciated layer of plant defence. By promoting beneficial microbes and enhancing stress resilience, this mechanism may help stabilize plant populations under anthropogenic pressures. Harnessing such interactions offers a promising path toward microbiome-informed strategies for sustainable agriculture and food system resilience.

Methods

Rhodotorula Correlation Network

Co-abundance network analysis was performed to investigate the interactions of *Rhodotorula* with other microbes in the phyllosphere microbiome of *Arabidopsis*. Operational Taxonomic Units (OTUs) were directly obtained from Mahmoudi et al.(2024)³⁰. The OTU table for all samples (epiphytes and endophytes), including bacterial and eukaryotic (fungal and nonfungal) OTUs, was filtered to retain only those OTUs present in at least five samples with more than ten reads. SparCC³¹ correlations were computed using the FastSpar³² platform with default parameters. Permuted *p*-values for each correlation were derived from 1,000 bootstrap datasets, and only correlations with $P \leq 0.05$ were retained for further analysis. The co-abundance network was visualized using Cytoscape (version 3.10.0)³³. Nodes (OTUs) in the network were labelled at the genus level based on OTU assignments using the UNITE³⁴ database (version 02_02_2019) for fungi and the SILVA³⁵ database(version 138.1) for bacteria, as described in Mahmoudi et al. 2024³⁰.

Dropout co-cultivation of the “Core” SynCom Members

In this study, the SynCom, comprised of a diverse array of bacteria and yeast strains³⁶, was meticulously cultivated in co-culture assembled by dropping out of the members besides a full Syncom used as a co-culture positive control. Initially, strains were revived from 30% glycerol ($\geq 99.5\%$) cryo-stocks and cultivated on specific agar plates - Nutrient Broth (NB) agar for bacteria and Potato Dextrose Agar (PDA) agar for yeast. After incubating at 22 °C for 3 days, the strains were transitioned into liquid cultures, utilizing NB and Potato Dextrose Broth (PDB)

for bacteria and yeast, respectively, maintaining the same incubation conditions for an additional 2-3 days.

Following this, the bacteria and yeast liquid cultures were centrifuged at 4000 x g, and the resulting pellets were washed thrice with an MgCl₂ 10 mM solution. Subsequently, the strains were suspended in MgCl₂ 10 mM, yielding solutions with an optical density (OD) absorbance 1 OD. These solutions were then utilized to generate 16 distinct co-cultures, each excluding one of the 15 SynCom strains, a SynCom containing all members for comparative analysis was also included in these co-cultures. These co-cultures were inoculated onto minimal medium (MM9) agar plates and allowed to grow at 22 °C for 5 days. After that, microbial cultures were harvested by carefully scraping cells from the plates.

Amplicon sequencing of in-vitro SynCom communities with dropout members

DNA was extracted using the ZymoBIOMICS DNA Miniprep Kit (Zymo Research Corp., Irvine, CA, USA) following the manufacturer's protocol. For bacterial community characterization, 16S amplicons of the V3-V8 region (~1,300 bp) were generated by PCR using primers 341F³⁷ and 1378R³⁸ based on the protocol previously described by Seol et al³⁹. Reactions for generating 16S amplicons were carried out with 50 ng of DNA in 11.5 µL of nuclease-free water, 0.5 µL of each primer and 12.5 µL of LongAMP Taq 2x Master Mix (New England Biolabs, Ipswich, MA, USA) for a total reaction volume of 25 µL. The PCR cycle program was: 1 minute 95 °C; 25 cycles of 20 s 95 °C, 30 seconds at 55 °C, 120 seconds at 65 °C and a final elongation time of 5 minutes at the same temperature. For fungi characterization, ITS amplicons were generated using primers ITS1 TCCGTAGGTGAACCTGCGG and ITS4 TCCTCCGCTTATTGATATGC⁴⁰. Reactions for generating ITS amplicons were carried out with 50 ng of DNA in 11.5 µL of nuclease-free water, 0.5 µL of each primer and 12.5 µL of LongAMP Taq 2x Master Mix (New England Biolabs, Ipswich, MA, USA) for a total reaction volume of 25 µL. The PCR cycle program was: 1 minute 95 °C; 25 cycles of 15 seconds 95 °C, 15 seconds at 55 °C, 30 seconds at 65 °C and a final elongation time of 2 minutes at the same temperature. After PCR, Amplicons were purified using AMPure XP beads (Beckman Coulter, Brea CA, USA) at a 0.6 x bead-to-sample ratio and quantified with a Qubit 4 fluorometer. Nanopore sequencing libraries were prepared with the Rapid Barcoding Kit (SQK-RBK114.96) using the Amplicon sequencing from DNA protocol (available at <https://nanoporetech.com/document/rapid-sequencing-v14-amplicon-sequencing-sqk-rbk114-24-or-sqk>) and sequenced on MinION V14 flow cells (Oxford Nanopore Technologies) using MinKNOW v25.05.14. Only reads with a quality score ≥10 were retained and demultiplexed. Taxonomic classification was performed using Emu v3.4.5⁴¹ with a custom

rRNA/ITS operon reference database generated from the species genomes using the in silico PCR implemented in EMBOSS-primersearch⁴² as previously reported³⁹.

LC-MS/MS non-targeted metabolomic analysis

The extraction of metabolites involved the addition of an 80% methanol solution (LC/MS grade, Fisher Scientific) to the microbial cultures. The solutions were then vortexed for 2 minutes followed by ultrasonication for 15 minutes in an ultrasonicator bath. Subsequently, debris was removed from the solution through centrifugation at 13000 x g for 20 minutes.

Quantification of metabolites was achieved by evaporating the solution in pre-weighted vials. To create a 5 mg/mL concentration, the dried metabolites were dissolved in a 50% methanol solution. Non-target metabolomics was performed by liquid chromatography-tandem mass spectrometry (LC-HRMS/MS) with a UHPLC coupled to a Q-Exactive HF mass spectrometer. In short, the UHPLC separation was performed using a C18 core-shell column (Kinetex, 50 × 2.1 mm, 1.7 µm particle size, 100 Å pore size, Phenomenex, Torrance, USA). The mobile phases used were solvent **a**, H₂O (LC/MS grade, Fisher Scientific) + 0.1% formic acid (FA) and solvent **b**, acetonitrile (LC/MS grade, Fisher Scientific) + 0.1% FA. After the sample injection, a linear gradient method of 10 min was used to elute small molecules, and the flow rate was set to 500 µL/minute (normal flow). The following separation conditions were set: in the time range 0 - 8 minutes from 5% to 50% solvent **b**, was used, 8 – 10 minutes from 50 to 99% B, followed by 3 minute washout phase at 99% B and 3 minute re-equilibration phase at 5% B.

The measurements were conducted in positive mode, the Heated Electrospray Ionization (HESI) parameters included a sheath gas flow rate of 50 L/minute, auxiliary gas flow rate of 12 L/minute, and sweep gas flow rate of 1 L/minute. The spray voltage was set to 3.50 kV and the inlet capillary temperature to 250 °C, while the S-lens RF level to 50 V and the auxiliary gas heater temperature to 400 °C. The full MS survey scan acquisition range was set to 120–1,800 m/z with a resolution of 45,000, automatic gain control (AGC) of 1E6, and maximum injection time of 100ms with one micro-scan. DDA MS/MS spectra acquisition was performed in data-dependent acquisition (DDA) mode with TopN set to (5) as a consequence the five most abundant precursor ions of the survey MS scan were destined for MS/MS fragmentation. The resolution of the MS/MS spectra was set to 15,000, the AGC target to 5E5, and the maximum injection time to 50 ms. The isolation window for the precursor ion selection was set to 1.0 m/z and the normalized collision energy was set to a stepped increase from 25 to 35 to 45. MS/MS experiments were triggered at the apex of peaks within 2–15 seconds from their first occurrence. Dynamic exclusion was set to 5 seconds⁴³.

Raw data were converted into centroid mode file mzML, and uploaded on MZmine⁴⁴ for spectra processing. Briefly, the mass levels of noise were set at 3.0E5 and 1.0E4 for the mass detection at MS1 and MS2, respectively. The ADAP Chromatogram Builder Module was used with at least 5 consecutive scans needed for the peak definition and maximum mass tolerance set at 10 ppm. To achieve the feature resolving the ms/ms scan pairing and the retention time dimension were activated. In addition, the isotopic peak finder and the join aligner modules were applied together with the export modules for FBMN (batch file in the SI). Feature table and mgf file were used to create FBMN on the GNPS2 platform⁴⁵. FBMN task ID was used for data uploading on FBMN-STAT for data preparation, including blank removal, imputation, and normalization⁴⁶. Statistical analyses were conducted using custom Python code in Jupyter Notebook.

Native metabolomics with the metal infusion

To investigate the dynamics of siderophore production within the SynCom, we followed the method established by Aron et al. (2022)⁴⁷. Extracts from the dropout co-culture experiments, conducted in biological triplicate, were analysed to detect the presence of iron-binding metabolites, across all 15 dropout co-cultures. The analysis employed a two-step native ESI-LC-MS/MS workflow, which involved post-column infusion of ammonium acetate buffer at an initial concentration of 100 mM for post-pH adjustment, followed by the infusion of Iron(III) chloride hexahydrate solution at an initial concentration of 3 mM. Data acquisition was performed in data-dependent acquisition (DDA) mode, adhering to the parameters outlined in the LC-MS/MS non-targeted metabolomic analysis section described previously. Data analysis was conducted using the ion identity molecular networking (IIMN) workflow to observe the iron-bound adduct, in combination with MZmine4 and the GNPS platform. The converted raw data files coming from the metal infusion “Off” samples in centroid mode (mzML format) were additionally utilized for in silico siderophore detection. MassQL queries were formulated and used in the workflow called “massql_workflow” available on the GNPS2 platform⁴⁸. This allows us to identify iron-bound small molecules within our dataset. The queries considered both single-charged and double-charged iron masses. Cytoscape (version 3.7.1)³³ was used to visualize molecular networks.

Leaf-associated Bacteria Isolation and Characterization

Commensal *Pseudomonas* strains from the *Arabidopsis* leaf-associated culture collection (Kemen laboratory), stored in 30% glycerol ($\geq 99.5\%$) at $-80\text{ }^{\circ}\text{C}$, were used in this study. These strains were previously subcultured and confirmed for purity. The 16S rRNA gene was PCR-amplified, sequenced, and taxonomically assigned via BLAST against the NCBI Type

Strain database. Pathogenic *Pseudomonas* strains were provided by the Weigel laboratory (Max Planck Institute for Biology, Tübingen), and their genome sequences are publicly available.

Whole Genome Sequencing (WGS) and taxonomy of *Pseudomonas* strains

Commensal *Pseudomonas* leaf-associated bacteria were cultured in NB broth and genomic DNA from each was isolated, and the quality of the genomic DNA was assessed using agarose gel electrophoresis. DNA concentrations were estimated using a Nanodrop spectrophotometer ND-100 (Thermo Fisher Scientific, USA) and Quantus™ Fluorometer (Promega). Both the ratio A260/280 and gel electrophoresis were used to ascertain the quality and purity of DNA samples. The input of 1 µg of genomic DNA was used for library construction and sequencing of libraries Illumina platform (Illumina, San Diego, CA) using a NovaSeq X Plus, 2 x 151 bp* paired-end kit in the sequencing facility in CeGaT (Tübingen) (**Supplementary Table 2**).

Raw sequencing reads of *Pseudomonas* strains were first demultiplexed and subjected to quality control and adapter trimming using fastp (v 0.23.4) ⁴⁹ with default parameters. The trimmed reads were then assembled de novo using SPAdes (v 4.0.0) ⁵⁰. Genome annotation was performed using Bakta (v 1.9.4) ⁵¹ with database version 5.1. Taxonomic classification of the assembled genomes was conducted using GTDB-Tk (v 2.6.1) ⁵² against GTDB database release r226. Functional annotation of identified CDSs was done using eggNOG-mapper (v 2.1.12) ⁵³ in DIAMOND mode.

Pangenome analysis

To analyse the pangenome of the assembled genomes of the isolates, as well as the reference genomes of *P. atacamensis* Jug 3.2 (GCF_022802935.1), *P. koreensis* (GCF_001654515.1), *P. syringae* (GCF_018394375.1), and *P. viridiflava* (GCF_900184295.1) in anvi'o, raw genome assemblies were first reformatted keeping contigs of at least 2,500 bp. Several annotation steps were performed, including the assignment of functional annotations with NCBI COGs (anvi-run-ncbi-cogs), KEGG ortholog annotations (anvi-run-kegg-kofams), PFAM domain identification (anvi-run-pfams). The pangenome analysis was refined using an MCL inflation value of 10 and a minimum bit score ratio of 0.5, with NCBI BLAST as the sequence similarity search tool. Additional metadata regarding the pathogenicity of the isolates was added to the anvi'o pangenome analysis, with *P. viridiflava* and *P. syringae* strains marked as positive for pathogenicity and the rest marked negative. Finally, functional enrichment analyses were conducted using anvi-compute-functional-enrichment-in-pan, focusing on

genes associated with pathogenicity based on COG20_FUNCTION and COG20_PATHWAY annotations, and the results were visualized using anvio in interactive mode.

The gene-enrichment analysis of the pathogenic-vs-commensal strains revealed different clustering of COG4773 (FhuE) and COG4774 (Fiu) functions. To investigate these further, we build a more comprehensive pangenome of all representative species available in the GTDB database, using anvio as described above. The presence-absence of gene clusters associated with these functions were then visualized using iTOL, projected onto the subtree of the genus *Pseudomonas_E* from GTDB.

In order to further investigate the functional diversity of TonB-related protein in *P. viridiflava* strains, we screened for proteins containing the PFAM domains PF00593.30 (TonB dependent receptor-like, beta-barrel), PF07660.19 (Secretin and TonB N terminus short domain (STN)), or PF07715.20 (TonB-dependent Receptor Plug Domain) in all publicly available *P. viridiflava* genome assemblies (1396 genomes from GTDB). We collected the respective HMM models from PFAM and searched them against *P. viridiflava* protein sequences using hmmscan and extracted all the proteins that hit a combination of these domains (6051 proteins). We generated a multiple sequence alignment of these proteins using MAFFT (v 7.526, with parameters --localpair --maxiterate 1000)⁵⁴, and followed by a maximum likelihood tree construction using FastTree (v 2.1.11, with parameters --gamma)⁵⁵.

Construction of *Pseudomonas koreensis* knockout strains

Chromosomal deletions were performed by single homologous recombination using two plasmids with different antibiotic cassettes. Briefly, 530 bp of fpvB (RS21125), and 597 bp of fiuA (RS16190) were cloned into pCR 2.1 and, pJQ200KS plasmids, respectively (Invitrogen). Cloning into pCR 2.1 was performed according to manufacturer recommendations while restriction enzymes and T4-DNA ligase were used for the construct (BamHI-XhoI for pJQ200KS-fiuA). The oligonucleotides used are shown in below. The resulting plasmids were confirmed by PCR, restriction pattern and sequencing, and then electroporated into *Pseudomonas koreensis*. Selection was performed using the corresponding antibiotic resistances (Km, and Gm respectively). Resistant clones were checked by PCR to confirm mutation in the target genes. Once single mutants were obtained, we next performed double mutants by cloning plasmids into single mutant strains following the same procedure described above.

Oligos:

FiuA_PJQ_fwd GGATCCCACCAAACCTCACTCTGCTGAGCCAGTTCAC

FiuA_PJQ_rev CTCGAGGTCGAGAGCCATTTGGTCCTGCACGTAGAG

585 Fpvh_pCR2.1_fwd TCACGGCCTATTCTACGCCATCGGTGAAGC

586 Fbvh_pCR2.1_rev GACTTGCCGCTACGCACGAAGTCAGGCTTG

587

588 ***SynCom and Pseudomonas growth curves under Rhodotorula supernatant***

589 The 15 SynCom member strains were used to generate individual growth curves in the
590 presence of *Rhodotorula* culture supernatant. Treatment samples consisted of a 1:1 (v/v)
591 mixture of supernatant from a *Rhodotorula* strain cultured for 5 days in MM9 medium and fresh
592 MM9 medium; fresh MM9 medium alone was used as the control. MM9 minimal medium was
593 prepared as described previously ³⁶. After autoclaving the basal salts MgSO₄ and CaCl₂,
594 glucose, a defined amino acid mixture (17 essential amino acids), and a trace element solution
595 were added under sterile conditions after autoclaving. Experiments were performed in 96-well
596 plates using a Tecan plate reader. Optical density at 600 nm (OD₆₀₀) was measured every 30
597 minutes for 24–48 hours at 22 °C with continuous shaking at 125 rpm.

598 Rhodotorulic acid, a siderophore present in the *Rhodotorula* supernatant, was detected and
599 quantified by using LC/MS-MS analysis. The *Rhodotorula* exometabolome was isolated by
600 solid-phase extraction (SPE), and 400 µL of the crude extract, concentrated to 5 mg/mL, was
601 subjected to fractionation. A rhodotorulic acid standard was obtained from Biophore Research
602 Products (Rottenburg, Germany) and was used as a positive control to assess its effect on
603 bacterial growth through growth-curve experiments. Biological replicates were included for all
604 experiments.

605 The contribution of rhodotorulic acid to the growth promotion of SynCom *Pseudomonas* strains
606 was evaluated, and additional growth-curve assays were performed using multiple plant-
607 associated *Pseudomonas* strains in the presence of *Rhodotorula* supernatant.

608 To check if *Pseudomonas* grows in varying nutrient conditions and rhodotorulic acid
609 supplementation, growth curves were performed in MM9 medium, which served as the control
610 condition, and seven additional treatments were tested by omitting glucose and/or the amino
611 acid mixture and by supplementing the media with rhodotorulic acid (50 µg/mL). All media
612 were prepared fresh and used for growth curve measurements under identical experimental
613 conditions.

614

615

616 ***In-planta protection evaluation assays***

617

618 For the greenhouse experiments, seeds of *Arabidopsis thaliana* (genotype WS-0) were sown

and allowed to germinate in soil under short-day conditions (10 hours of light at 21 °C and 14 hours of darkness at 16 °C). After one month, for non-sterile plants, the resulting seedlings were individually transplanted into 6-pot trays and cultivated under short-day conditions. Adult plants were divided into experimental groups and treated with the SynCom strains *Pseudomonas*, *Rhodotorula*, or a combination of both. Each treatment was applied by spraying the leaves with a microbial suspension prepared in 10 mM MgCl₂. Control plants were sprayed with 10 mM MgCl₂ solution without microbial inoculation. After five days of microbial establishment on the leaves, both treated and control plant groups were inoculated with *Pseudomonas syringae* pv. *tomato* DC3000. Disease progression was assessed five days after pathogen inoculation by counting the number of symptomatic leaves relative to the total number of leaves per plant.

Gnotobiotic plant experiments were performed to investigate the effects of *Pseudomonas*, *Rhodotorula*, the siderophore rhodotorulic acid (RA), and their combinations on the host, as well as to assess SynComs and SynCom variants containing *Pseudomonas* knockout strains. Experiments were conducted using *Arabidopsis thaliana* WS-0 genotype seeds, which were surface-sterilized prior to use. Seeds were germinated on plant agar plates under short-day conditions (10 hours light at 21 °C, 14 hours dark at 16 °C). Seedlings of uniform size were subsequently transferred individually into 12-well plates for further growth and treatment. Sterile split dishes were used to physically separate the aerial parts of the plants from the agar medium containing the root system. The treated groups received a foliar spray containing a suspension of the 15 strains comprising the SynCom. In the respective treatment groups, the *Pseudomonas* member (wild type) was replaced with the knockout variants (*fpvB*⁻, *fiuA*⁻, or *fpvB*⁻ *fiuA*⁻). Each strain was adjusted to an OD₆₀₀ of 0.2 in 10 mM MgCl₂ before application. Control plants were sprayed with 10 mM MgCl₂ solution alone. Pathogen inoculation with *P. syringae* pv. *tomato* DC3000 for all groups was performed five days after the SynCom treatment to allow for microbial establishment on the phyllosphere. Plates containing plants were maintained under short-day conditions. Gnotobiotic plants were then assessed for disease symptoms as described for the greenhouse experiments, by quantifying the percentage of symptomatic leaves over the total. In addition, *P. syringae* quantification was determined by counting colony-forming units (CFUs) per gram of fresh weight (FW). The epiphytic microbial community was collected for amplicon sequencing, and additional plants were harvested for metabolite extraction and subsequent high-resolution tandem mass spectrometry (LC-MS/MS) analysis.

Amplicon sequencing of the epiphytic synCom community

Arabidopsis thaliana plants were grown gnotobiotically and inoculated with either the complete SynCom or derivative communities in which *Pseudomonas koreensis* 3.2 was replaced by knockout strains generated in this study, all suspended in 10 mM MgCl₂. Plants were harvested and briefly washed by shaking in 5 mL Milli-Q water for 10 seconds, after which the wash solution was discarded. Subsequently, 5 mL of epiphyte wash solution (1 × TE + 0.1% Triton X-100) was added, and the plants were gently shaken for 1 minute. Epiphytic microorganisms were collected by transferring the resulting suspension into syringe barrels mounted in filter holders. After reinserting the plungers, the suspension was filtered through 0.22 µm mixed cellulose ester (MCE) membrane filters. The filter holders were then opened, and the filters were carefully retrieved and stored in 2mL screw-cap tubes. The retrieved membrane filters were frozen in liquid nitrogen and mechanically disrupted by cryogenic lysis. Samples were processed at 6300 rpm for four 30 second cycles with 15 second pauses between cycles, yielding a finely homogenized material for subsequent extraction steps.

DNA was extracted using the same approach reported above. Genomic DNA was quantified with a Qubit dsDNA BR/HS Assay Kit (Thermo Fisher) and normalized to 5-25 ng Input for library preparation. Library preparation was performed as a two-step PCR approach. The first step PCR for the 16S rRNA V4 region was performed in 15 µl reactions including KAPA HiFi HotStart ReadyMix (Roche), 515F and 806R (Parada et al 2016; April et al 2015) and template DNA (PCR program: 95 °C for 3 minutes, 30x (98 °C for 20 seconds, 55 °C for 15 seconds, 72 °C for 15 seconds), 72 °C for 5 minutes). While the first step PCR for the ITS1 region was performed in 15 µl reactions including KAPA HiFi HotStart ReadyMix (Roche), ITS1F and ITS2R (Gardes and Bruns 1993; White et al. 1990) and template DNA (PCR program: 95 °C for 3 minutes, 35x (98 °C for 20 seconds, 50 °C for 1 minute, 72 °C for 90 seconds), 72 °C for 5 minutes).

PCR products were purified using 12 µl AMPure XP beads and eluted in 26 µL 10 mM Tris-HCl. Indexing for both target regions was performed in the second step PCR including KAPA HiFi HotStart ReadyMix (Roche), index primer mix (IDT® for Illumina Nextera DNA Unique Dual Indexes), purified first PCR product as template (PCR program: 95°C for 3 min, 8x (95°C for 30 sec, 55°C for 30 sec, 72°C for 30 sec), 72°C for 5 min). After another bead purification (14 µl AMPure XP beads, eluted in 15 µL 10mM Tris-HCl) the libraries were checked for correct fragment length on an agarose gel, quantified with a Qubit dsDNA BR Assay Kit (Thermo Fisher) and pooled equimolarly. The pool was sequenced on an Illumina Miseq with a v3 sequencing kit and 2x300 bp read length and a depth of 40-140k reads per sample.

Raw sequencing reads were demultiplexed and quality-trimmed using QIIME2 (Version 2024.10)⁵⁶. Trimming parameters were selected that the average phred score across retained

bases exceeded 30 for both 16S and ITS amplicons forward and reverse reads. Amplicon sequencing variants (ASVs) were inferred using DADA2 Pipeline implemented in QIIME2⁵⁷.

Taxonomic classification was performed using QIIME2 feature-classifier⁵⁸ with a custom rRNA/ITS operon reference database constructed from the genomes of the species used in this study. Chloroplast and low abundant ASVs (fewer than 50 total reads and present in more than four samples) were removed using phyloseq R package⁵⁹. ASV sequences that could not be classified on genus level were subsequently submitted to BLAST to enhance taxonomic resolution. Finally, relative abundances were calculated based on filtered ASV tables using phyloseq.

***Pseudomonas syringae* CFU-determination from gnotobiotic in planta experiments**

For bacterial quantification, plant material was homogenized in 10 mM MgCl₂ using zirconia beads. Plants were transferred to pre-weighed tubes containing 1 mL MgCl₂ and a mixture of 3-, 2-, 0.5-, and 0.1- mm zirconia beads, and fresh weight was determined. Samples were lysed using a Precellys homogenizer (four cycles at 6300 rpm for 30 seconds with 15 second pauses). Homogenates were serially diluted in MgCl₂ in 96-well plates, and aliquots were plated onto NB agar supplemented with rifampicin and cycloheximide. Rifampicin was used to selectively recover *Pseudomonas syringae*, which is resistant to the antibiotic, whereas other bacterial community members are not, and cycloheximide was included to suppress the growth of yeasts and filamentous fungi. Plates were incubated at room temperature, and colonies for each dilution were counted after 24 and 48 hours to calculate colony-forming units per millilitre.

Funding:

This project was supported by the European Union's Horizon Europe research and innovation programme through a Marie Skłodowska-Curie fellowship MeStaLeM, n. 101108450. PS, NZ, and DP were supported by the German Research Foundation through the Cluster of Excellence EXC 2124: Controlling Microbes to Fight Infection (CMFI, project ID 390838134). CB and NZ were supported by the German Center for Infection Research (TTU09.716) and the Momentum Funding of the Volkswagen Foundation. A.I.P.L. is funded by the program FPU(FPU19/00289) and the program Plan Propio de Investigación y Transferencia from Universidad de Málaga. CMS is funded by grants from Consolidación Investigadora (CSN2022-135744) and Agencia Estatal de Investigación of the Ministerio de Ciencia e

Innovación (PID2024-156932NA-I00). ATA was supported by National Institutes of Health (R35GM155026).

Data availability:

All data needed to evaluate the conclusions in the paper are present in the paper or the Supplementary Materials. Raw amplicon sequencing data have been deposited in the NCBI Sequence Read Archive (SRA) under BioProject PRJNA1413978. Whole-genome sequencing data and the corresponding genome assemblies are available in the European Nucleotide Archive (ENA) under project accession PRJEB106563. Metabolomics raw data (previously centroided mzML MS/MS files) are deposited on Zenodo under DOI 10.5281/zenodo.18199851. Pangenome analysis datasets and all analysis code generated in this study are publicly available in the same Zenodo repository.

References

1. Vorholt, J. A. Microbial life in the phyllosphere. *Nat Rev Microbiol* **10**, 828–840 (2012).
2. Berg, G. *et al.* Microbiome definition re-visited: old concepts and new challenges. *Microbiome* **8**, 103 (2020).
3. Compant, S., Samad, A., Faist, H. & Sessitsch, A. A review on the plant microbiome: Ecology, functions, and emerging trends in microbial application. *Journal of Advanced Research* **19**, 29–37 (2019).
4. Trivedi, P., Leach, J. E., Tringe, S. G., Sa, T. & Singh, B. K. Plant–microbiome interactions: from community assembly to plant health. *Nat Rev Microbiol* **18**, 607–621 (2020).
5. Xu, L. *et al.* Genome-resolved metagenomics reveals role of iron metabolism in drought-induced rhizosphere microbiome dynamics. *Nat Commun* **12**, 3209 (2021).
6. Laine, A.-L. & Leino, S. Plant microbiomes feel the heat. *Science* **388**, 1150–1152 (2025).
7. Ge, A.-H. & Wang, E. Exploring the plant microbiome: A pathway to climate-smart crops. *Cell* **188**, 1469–1485 (2025).

8. Chen, T. *et al.* A plant genetic network for preventing dysbiosis in the phyllosphere. *Nature* **580**, 653–657 (2020).
9. Su, P. *et al.* Microbiome homeostasis on rice leaves is regulated by a precursor molecule of lignin biosynthesis. *Nat Commun* **15**, 23 (2024).
10. Shalev, O. *et al.* Commensal *Pseudomonas* strains facilitate protective response against pathogens in the host plant. *Nat Ecol Evol* **6**, 383–396 (2022).
11. Shalev, O., Ashkenazy, H., Neumann, M. & Weigel, D. Commensal *Pseudomonas* protect *Arabidopsis thaliana* from a coexisting pathogen via multiple lineage-dependent mechanisms. *The ISME Journal* **16**, 1235–1244 (2022).
12. Hider, R. C. & Kong, X. Chemistry and biology of siderophores. *Nat. Prod. Rep.* **27**, 637 (2010).
13. Kramer, J., Özkaya, Ö. & Kümmerli, R. Bacterial siderophores in community and host interactions. *Nat Rev Microbiol* **18**, 152–163 (2020).
14. Almario, J. *et al.* The Leaf Microbiome of *Arabidopsis* Displays Reproducible Dynamics and Patterns throughout the Growing Season. *mBio* **13**, e02825-21 (2022).
15. Gómez-Pérez, D. *et al.* Proteins released into the plant apoplast by the obligate parasitic protist *Albugo* selectively repress phyllosphere-associated bacteria. 2022.05.16.492175 Preprint at <https://doi.org/10.1101/2022.05.16.492175> (2022).
16. Will, V. *et al.* The role of FoxA, FiuA, and FpvB in iron acquisition via hydroxamate-type siderophores in *Pseudomonas aeruginosa*. *Sci Rep* **14**, 18795 (2024).
17. Denness, L. *et al.* Cell Wall Damage-Induced Lignin Biosynthesis Is Regulated by a Reactive Oxygen Species- and Jasmonic Acid-Dependent Process in *Arabidopsis*. *Plant Physiology* **156**, 1364–1374 (2011).
18. Jin, C. W. *et al.* Iron Deficiency-Induced Secretion of Phenolics Facilitates the Reutilization of Root Apoplastic Iron in Red Clover. *Plant Physiol.* **144**, 278–285 (2007).
19. Liu, Q. *et al.* A lignin-derived material improves plant nutrient bioavailability and growth through its metal chelating capacity. *Nat Commun* **14**, 4866 (2023).

20. Fujita, M. *et al.* Functional roles of multiple Ton complex genes in a *Sphingobium* degrader of lignin-derived aromatic compounds. *Sci Rep* **11**, 22444 (2021).
21. Durán, P. *et al.* Microbial Interkingdom Interactions in Roots Promote Arabidopsis Survival. *Cell* **175**, 973-983.e14 (2018).
22. Zhan, C., Matsumoto, H., Liu, Y. & Wang, M. Pathways to engineering the phyllosphere microbiome for sustainable crop production. *Nat Food* **3**, 997–1004 (2022).
23. Karasov, T. L. *et al.* Arabidopsis thaliana and Pseudomonas Pathogens Exhibit Stable Associations over Evolutionary Timescales. *Cell Host & Microbe* **24**, 168-179.e4 (2018).
24. Spragge, F. *et al.* Microbiome diversity protects against pathogens by nutrient blocking. *Science* **382**, eadj3502 (2023).
25. Radlinski, L. C. & Bäumler, A. J. No room at the table. *Science* **382**, 1244–1245 (2023).
26. Quinn, R. A. *et al.* Niche partitioning of a pathogenic microbiome driven by chemical gradients. *Sci. Adv.* **4**, eaau1908 (2018).
27. Brader, G. *et al.* Ecology and Genomic Insights into Plant-Pathogenic and Plant-Nonpathogenic Endophytes. *Annu. Rev. Phytopathol.* **55**, 61–83 (2017).
28. Spanu, P. D. & Panstruga, R. Editorial: Biotrophic Plant-Microbe Interactions. *Front. Plant Sci.* **8**, (2017).
29. Van Der Does, D. *et al.* The Arabidopsis leucine-rich repeat receptor kinase MIK2/LRR-KISS connects cell wall integrity sensing, root growth and response to abiotic and biotic stresses. *PLoS Genet* **13**, e1006832 (2017).
30. Mahmoudi, M., Almario, J., Lutap, K., Nieselt, K. & Kemen, E. Microbial communities living inside plant leaves or on the leaf surface are differently shaped by environmental cues. *ISME Communications* **4**, ycae103 (2024).
31. Friedman, J. & Alm, E. J. Inferring Correlation Networks from Genomic Survey Data. *PLoS Comput Biol* **8**, e1002687 (2012).
32. Watts, S. C., Ritchie, S. C., Inouye, M. & Holt, K. E. FastSpar: rapid and scalable correlation estimation for compositional data. *Bioinformatics* **35**, 1064–1066 (2019).

33. Shannon, P. *et al.* Cytoscape: A Software Environment for Integrated Models of Biomolecular Interaction Networks. *Genome Res* **13**, 2498–2504 (2003).
34. Abarenkov, K. *et al.* The UNITE database for molecular identification and taxonomic communication of fungi and other eukaryotes: sequences, taxa and classifications reconsidered. *Nucleic Acids Research* **52**, D791–D797 (2024).
35. Quast, C. *et al.* The SILVA ribosomal RNA gene database project: improved data processing and web-based tools. *Nucleic Acids Research* **41**, D590–D596 (2012).
36. Höhn, F. *et al.* Strong pairwise interactions do not drive interactions in a plant leaf associated microbial community. *ISME Communications* **5**, ycae117 (2025).
37. Klindworth, A. *et al.* Evaluation of general 16S ribosomal RNA gene PCR primers for classical and next-generation sequencing-based diversity studies. *Nucleic Acids Research* **41**, e1–e1 (2013).
38. Leubhn, M. *et al.* Towards molecular biomarkers for biogas production from lignocellulose-rich substrates. *Anaerobe* **29**, 10–21 (2014).
39. Seol, D. *et al.* Microbial Identification Using rRNA Operon Region: Database and Tool for Metataxonomics with Long-Read Sequence. *Microbiol Spectr* **10**, e02017-21 (2022).
40. White, T. J., Bruns, T., Lee, S. & Taylor, J. AMPLIFICATION AND DIRECT SEQUENCING OF FUNGAL RIBOSOMAL RNA GENES FOR PHYLOGENETICS. in *PCR Protocols* 315–322 (Elsevier, 1990). doi:10.1016/B978-0-12-372180-8.50042-1.
41. Curry, K. D. *et al.* Emu: species-level microbial community profiling of full-length 16S rRNA Oxford Nanopore sequencing data. *Nat Methods* **19**, 845–853 (2022).
42. Rice, P., Longden, I. & Bleasby, A. EMBOSS: The European Molecular Biology Open Software Suite. *Trends in Genetics* **16**, 276–277 (2000).
43. Stincone, P. *et al.* Evaluation of Data-Dependent MS/MS Acquisition Parameters for Non-Targeted Metabolomics and Molecular Networking of Environmental Samples: Focus on the Q Exactive Platform. *Anal. Chem.* **95**, 12673–12682 (2023).
44. Heuckeroth, S. *et al.* Reproducible mass spectrometry data processing and compound annotation in MZmine 3. *Nat Protoc* **19**, 2597–2641 (2024).

45. Nothias, L.-F. *et al.* Feature-based molecular networking in the GNPS analysis environment. *Nat Methods* **17**, 905–908 (2020).
46. Pakkir Shah, A. K. *et al.* Statistical analysis of feature-based molecular networking results from non-targeted metabolomics data. *Nat Protoc* **20**, 92–162 (2025).
47. Aron, A. T. *et al.* Native mass spectrometry-based metabolomics identifies metal-binding compounds. *Nat. Chem.* **14**, 100–109 (2022).
48. Damiani, T. *et al.* A universal language for finding mass spectrometry data patterns. *Nat Methods* **22**, 1247–1254 (2025).
49. Chen, S., Zhou, Y., Chen, Y. & Gu, J. fastp: an ultra-fast all-in-one FASTQ preprocessor. *Bioinformatics* **34**, i884–i890 (2018).
50. Bankevich, A. *et al.* SPAdes: A New Genome Assembly Algorithm and Its Applications to Single-Cell Sequencing. *Journal of Computational Biology* **19**, 455–477 (2012).
51. Schwengers, O. *et al.* Bakta: rapid and standardized annotation of bacterial genomes via alignment-free sequence identification: Find out more about Bakta, the motivation, challenges and applications, here. *Microbial Genomics* **7**, (2021).
52. Chaumeil, P.-A., Mussig, A. J., Hugenholtz, P. & Parks, D. H. GTDB-Tk v2: memory friendly classification with the genome taxonomy database. *Bioinformatics* **38**, 5315–5316 (2022).
53. Cantalapiedra, C. P., Hernández-Plaza, A., Letunic, I., Bork, P. & Huerta-Cepas, J. eggNOG-mapper v2: Functional Annotation, Orthology Assignments, and Domain Prediction at the Metagenomic Scale. *Molecular Biology and Evolution* **38**, 5825–5829 (2021).
54. Katoh, K., Rozewicki, J. & Yamada, K. D. MAFFT online service: multiple sequence alignment, interactive sequence choice and visualization. *Briefings in Bioinformatics* **20**, 1160–1166 (2019).
55. Price, M. N., Dehal, P. S. & Arkin, A. P. FastTree 2 – Approximately Maximum-Likelihood Trees for Large Alignments. *PLoS ONE* **5**, e9490 (2010).

56. Bolyen, E. *et al.* Reproducible, interactive, scalable and extensible microbiome data science using QIIME 2. *Nat Biotechnol* **37**, 852–857 (2019).
57. Callahan, B. J. *et al.* DADA2: High-resolution sample inference from Illumina amplicon data. *Nat Methods* **13**, 581–583 (2016).
58. Bokulich, N. A. *et al.* Optimizing taxonomic classification of marker-gene amplicon sequences with QIIME 2's q2-feature-classifier plugin. *Microbiome* **6**, 90 (2018).
59. McMurdie, P. J. & Holmes, S. phyloseq: An R Package for Reproducible Interactive Analysis and Graphics of Microbiome Census Data. *PLoS ONE* **8**, e61217 (2013).

Acknowledgments:

We thank Libera Lo Presti for proofreading and helpful discussions. We also thank Abzer K. Pakkir Shah and Monja Schmid for their valuable suggestions. We thank the Weigel lab at the Max Planck Institute for Biology in Tübingen for kindly providing *Pseudomonas viridiflava* strains. We acknowledge support from the NGS Competence Center Tübingen (NCCT) and the Quantitative Biology Center (QBiC), University of Tübingen, Germany. ChatGPT-5 (OpenAI) was used for proofreading and text editing. All original content was authored by the authors, who take full responsibility for the manuscript.

Author information:

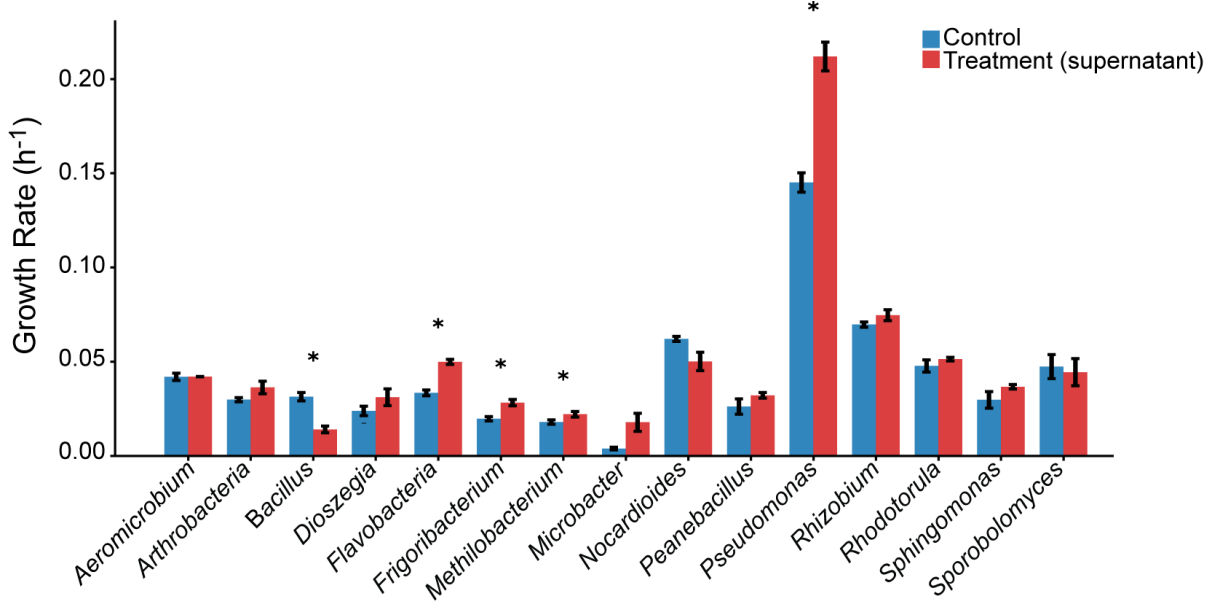
P.S., E.K., and D.P. conceptualized and designed the study and acquired funding. P.S. administered the project. P.S., L.B., C.B., M.N.D., A.P.L., S.F., J.B., K.S.L., M.M., V.C., D.R., N.Z., A.T.A., C.M.S., E.K., and D.P. performed the experiments and data analyses. P.S., E.K., and D.P. interpreted the data and wrote the manuscript. All authors revised the manuscript and approved the final version for submission.

Competing interests:

The authors declare that they have no competing interests.

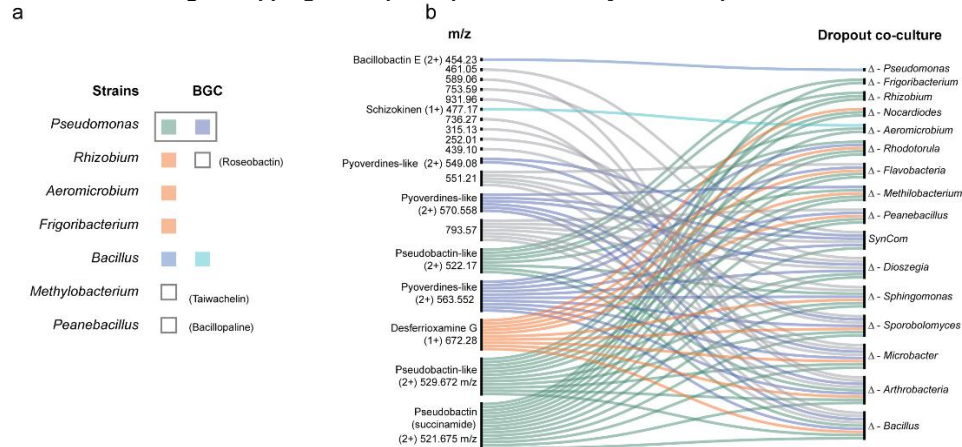
Extended data figures and tables

Extended Data Fig. 1 *Pseudomonas* growth is promoted by *Rhodotorula* supernatant.



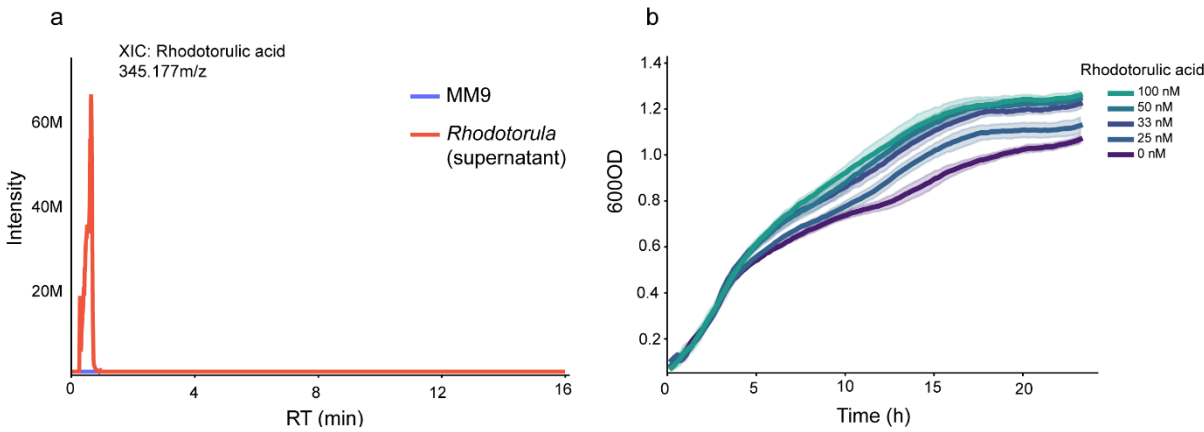
Bars show exponential growth rates of the 15 microbial strains comprising the phyllosphere core synthetic community (SynCom). Blue bars indicate growth in 100% fresh MM9 medium (control), whereas red bars indicate growth in a 50:50 (v/v) mixture of fresh MM9 and cell-free supernatant from a *Rhodotorula* preculture grown under iron-limited MM9 conditions, which stimulate RA production. Error bars denote standard deviation across replicate wells. Asterisks indicate significant differences between control and supernatant conditions (Welch's t-test, *P* < 0.05).

Extended Data Fig. 2 Mapping siderophore production in SynCom dropout co-cultures.



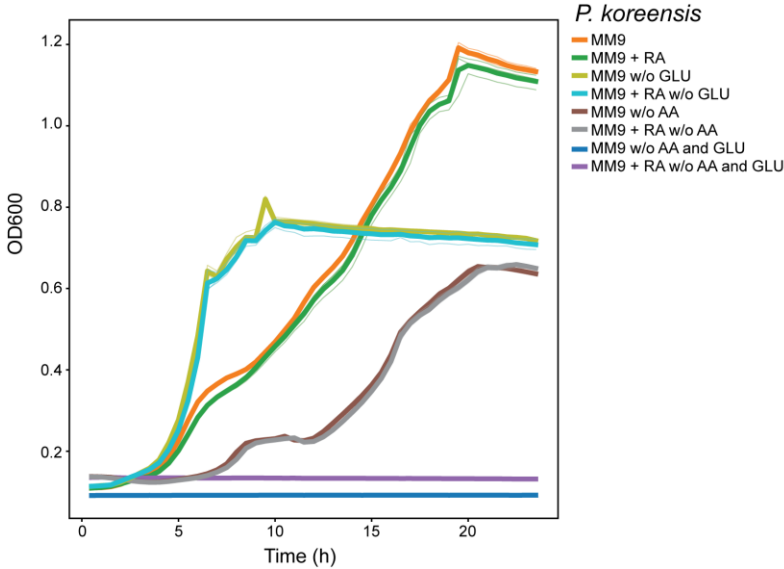
a, SynCom strains harboring biosynthetic gene clusters (BGCs) for siderophore production, annotated using antiSMASH. Strains are color-coded according to the corresponding siderophore masses reported in the alluvial plot, which depicts relationships between community members and their siderophore production profiles. **b**, Alluvial plot summarizing MassQL results, showing detected m/z values corresponding to query-targeted siderophore masses in both singly and doubly charged states. m/z values were extracted directly from raw mass spectrometry data and reflect the microbial cultures in which these features were detected. Siderophore biosynthesis, as with other community metabolites, is modulated by microbial interactions. In the SynCom, specific dropout co-culture conditions alter the capacity of individual community members to produce siderophores. Selected masses were validated by native metabolomics and Ion Identity Molecular Networking (IIMN) analysis, including the siderophore pseudobactin (m/z 521.675), whose apo-form was not visible in the IIMN cluster due to its high iron-chelating capacity, and desferrioxamine G (m/z 563.552).

Extended Data Fig. 3 Growth of *Pseudomonas koreensis* across decreasing rhodotorulic acid concentrations.



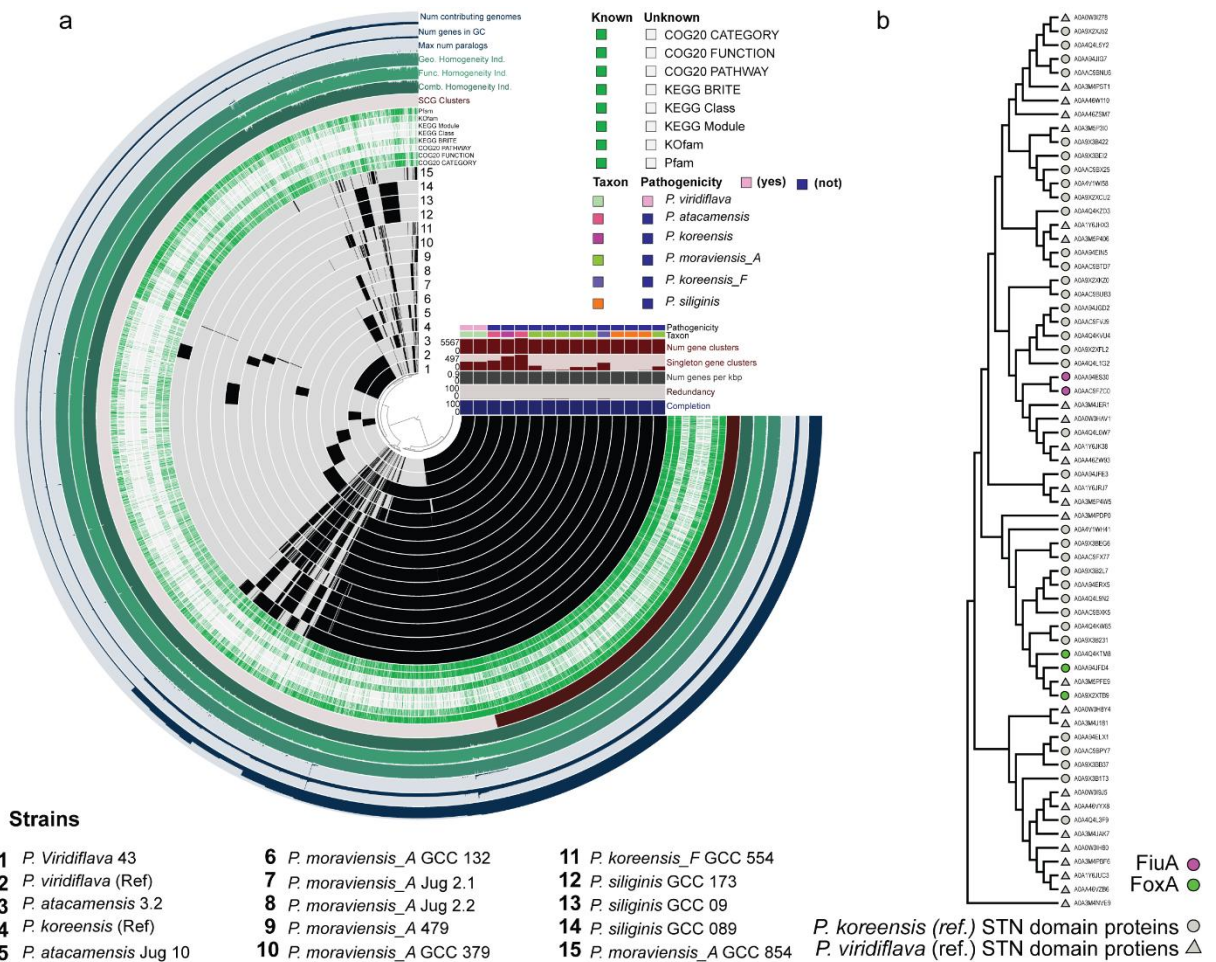
a, Extracted ion chromatogram (XIC) of rhodotorulic acid (345.177m/z) detected in the exometabolome of *Rhodotorula* culture supernatant. MM9 minimal medium served as a negative control and showed no detectable rhodotorulic acid signal. **b**, Growth curves of the SynCom *Pseudomonas koreensis* strain in MM9 minimal medium supplemented with decreasing concentrations of purchased rhodotorulic acid siderophore. MM9 without siderophore served as the control. Rhodotorulic acid enhanced growth, with maximal stimulation at ~100 nM; this effect progressively diminished at lower concentrations, consistent with concentration-dependent siderophore utilization for iron acquisition.

Extended Data Fig. 4 Growth of *Pseudomonas koreensis* under varying nutrient conditions and rhodotorulic acid supplementation.



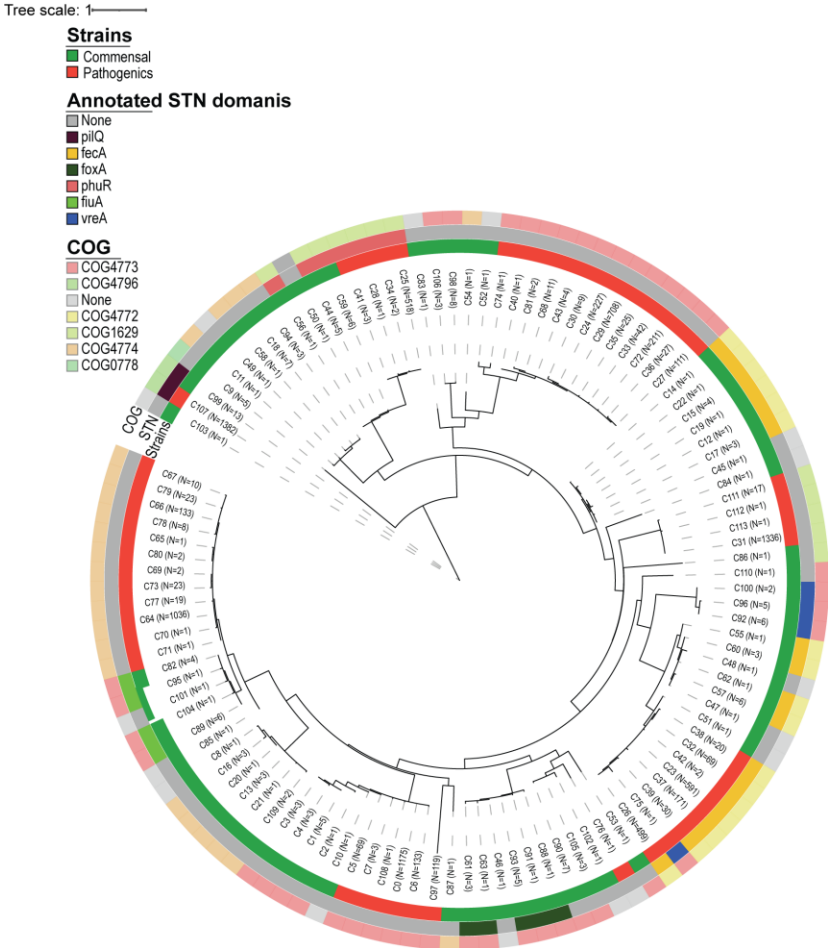
Growth curves of the SynCom *Pseudomonas koreensis* strain in MM9 minimal medium under the following conditions: standard MM9 $\pm$ rhodotorulic acid ($50 \mu\text{g mL}^{-1}$); MM9 lacking glucose $\pm$ rhodotorulic acid; MM9 lacking amino acids $\pm$ rhodotorulic acid; and MM9 lacking both glucose and amino acids $\pm$ rhodotorulic acid. Glucose depletion increased growth rate, whereas rhodotorulic acid supplementation did not support growth in carbon- or amino-acid-limited conditions, indicating it is not utilized as a carbon source.

937 Extended Data Fig. 5 Whole-genome sequencing comparison of plant-isolated strains.



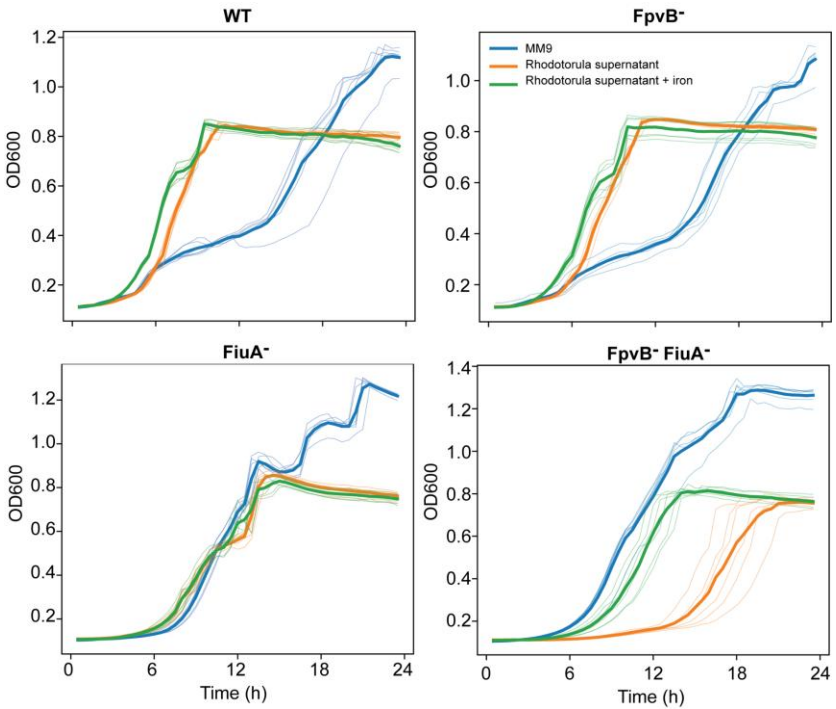
a, Phylogenetic relationships of plant-associated *Pseudomonas* isolates. Eleven commensal strains clustered with the *P. koreensis* reference genome, whereas the weakly pathogenic *P. viridiflava* strain P43 grouped with the reference genome of the same species. **b**, Phylogeny of STN-domain proteins from *P. koreensis* and *P. viridiflava* reference genomes. Protein sequences were annotated using COG and PFAM domains and used to construct a Maximum Likelihood tree, with initial topology generated by Neighbor-Joining using pairwise JTT distances. The analysis included 65 amino acid sequences.

Extended Data Fig. 6 Phylogenetic analysis of TonB-box amino acid sequences



A clear divergence between commensal strains sequenced in this study and publicly available *Pseudomonas viridiflava* genomes. The TonB-dependent receptors fluA and foxA are exclusively detected in the commensal strains.

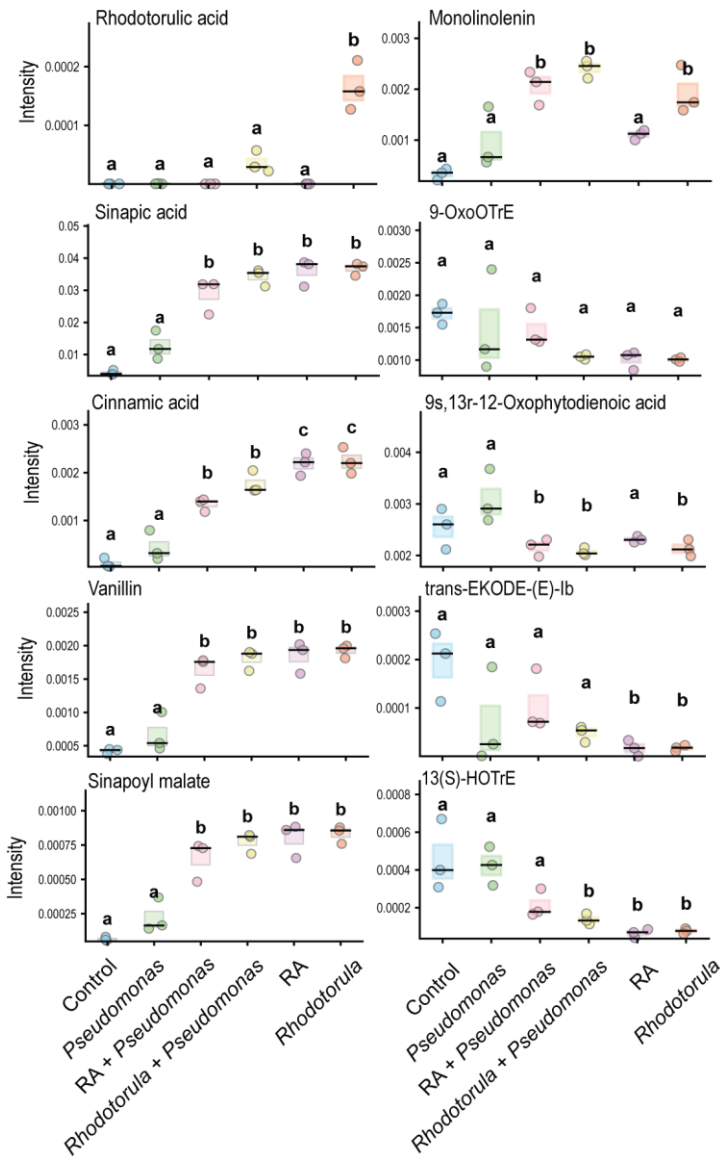
Extended Data Fig. 7 Iron limitation constrains the growth of *Pseudomonas koreensis* strains deficient in rhodotorulic acid uptake system.



Growth dynamics of *Pseudomonas koreensis* SynCom mutants lacking TonB-dependent transporters in MM9 minimal medium and in *Rhodotorula* culture supernatant, including conditions in which the supernatant was supplemented with an excess of iron to saturate the RA present in the supernatant. Impaired growth in the supernatant results from the absence of rhodotorulic acid transport and is rescued by iron supplementation, indicating that iron availability is a key determinant of growth in strains deficient in rhodotorulic acid uptake.

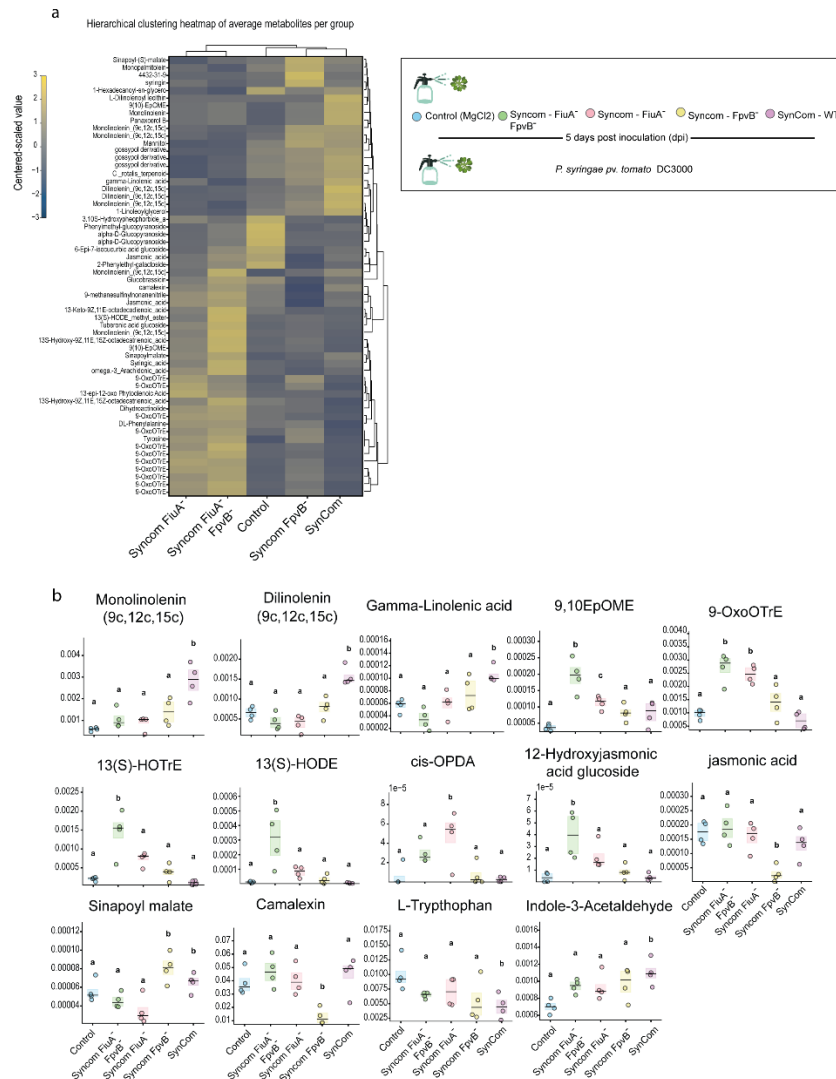
993

Extended Data Fig. 9 Differential regulation of lignin and jasmonic acid precursors during interkingdom interactions.



Gnotobiotic plants were pre-inoculated with *Pseudomonas*, *Rhodotorula*, the siderophore RA, or their combinations prior to infection with *Pseudomonas syringae*. Lignin precursors accumulation (Sinapic acid, Cinnamic acid, Vanillin, Sinapoyl malate) were associated with depletion of jasmonic acid precursors (9-OxoOTrE, 9s,13r-12-Oxophytodienoic acid, Trans Ekode e-Ib, 13(S)-HOTrE), particularly under Rhodotorula and RA treatment, consistent with reduced iron availability on leaf surfaces. One-way ANOVA ($P < 0.05$) followed by Tukey's HSD test; different letters indicate significant differences ($P < 0.05$).

Extended data Fig. 10 Non-targeted metabolomics of sterile plants exposed to SynCom variants containing substituted *Pseudomonas* strains.



a, Sterile *Arabidopsis thaliana* plants were treated with SynCom subsets in which the commensal *Pseudomonas* member was replaced by knockout strains. After 5 days of community establishment, plants were inoculated with *Pseudomonas syringae* and harvested 7 days post-infection. Centered and scaled intensities of significantly regulated GNPS-annotated metabolites were averaged by treatment and subjected to hierarchical clustering (average linkage, Euclidean distance). The heatmap displays metabolites (rows) and treatments (columns) ordered by similarity; colors indicate relative abundance (blue to yellow, -3 to +3). Dendrograms depict hierarchical relationships among metabolites and treatments. **b**, Polyunsaturated fatty acids and jasmonic acid (JA) and auxin precursors were differentially regulated in plants colonized by the full community, partly recapitulating patterns observed upon inoculation with *Pseudomonas* and *Rhodotorula* alone. This trend was inverted in communities containing knockout strains, with increased JA precursors and reduced polyunsaturated fatty acids. Among phenolic, lignin-associated metabolites, only sinapoyl malate was differentially regulated, showing increased abundance in SynComs containing either the wild-type or the Fpvb mutant strain. One-way ANOVA ($P < 0.05$) followed by Tukey's HSD test; different letters indicate significant differences ($P < 0.05$).

1018

Name	scan1	m/z	GNPS library	scan2	m/z	GNPS library	EdgeAnnotation
85917 (-) 85951	85917	518.32401	PC(18:3/0:0); [M+H] ⁺ C26H49N1O7P1	85951	286.6249	#N/A	[M+Fe+2] ⁺ +2 [2M+2H] ⁺ +2 dm/z=231.69907
85884 (-) 85951	85884	1035.6385	#N/A	85951	286.6249	#N/A	[M+Fe+2] ⁺ +2 [2M+H] ⁺ dm/z=749.01351
89758 (-) 89842	89758	480.30827	Spectral Match to 1-(9Z-Octadecenyl)- sn-glycero-3- phosphoethanolamine from NIST14	89842	267.6173	#N/A	[M+Fe+2] ⁺ +2 [M+H] ⁺ dm/z=212.69094
90415 (-) 90241	90415	288.64064	#N/A	90241	522.3553	Spectral Match to 1-(9Z-Octadecenyl)-sn-glycero-3-phosphocholine from NIST14	[M+Fe+2] ⁺ +2 [M+H] ⁺ dm/z=233.71462
104651 (-) 104701	104651	324.28943	#N/A	104701	378.2088	#N/A	[M+Fe+2-H] ⁺ [M+H] ⁺ dm/z=53.91933
10623 (-) 11099	10623	689.34642	#N/A	11099	398.0882	#N/A	[M+Fe+3-2H] ⁺ [2M+H] ⁺ dm/z=291.25819
10848 (-) 11099	10848	345.17671	Rhodotorulic acid	11099	398.0882	#N/A	[M+Fe+3-2H] ⁺ [M+H] ⁺ dm/z=52.91153
32289 (-) 33426	32289	619.36629	Desferrioxamine G	33426	336.6423	#N/A	[M+Fe+3-H] ⁺ +2 [M+H] ⁺ dm/z=282.72401
36653 (-) 36511	36653	420.04145	#N/A	36511	786.1647	FLAVIN ADENINE DINUCLEOTIDE	[M+Fe+3-H] ⁺ +2 [M+H] ⁺ dm/z=366.12326
96862 (-) 97137	96862	610.36758	#N/A	97137	279.2317	Spectral Match to 9(10)- EpOME from NIST14	[M+H] ⁺ [2M+Fe+3-2H] ⁺ dm/z=331.13592
9952 (-) 10848	9952	742.25815	#N/A	10848	345.1767	Rhodotorulic acid	[M+H] ⁺ [2M+Fe+3-2H] ⁺ dm/z=397.08144
97068 (-) 96862	97068	261.22113	#N/A	96862	610.3676	#N/A	[M-H2O+H] ⁺ [2M+Fe+3- 2H] ⁺ dm/z=349.14645
104502 (-) 104361	104502	325.27343	#N/A	104361	396.1954	#N/A	[M-H2O+H] ⁺ [M+Fe+3- 2H] ⁺ dm/z=70.92199
104361 (-) 104523	104361	396.19542	#N/A	104523	342.3	#N/A	[M-H2O+NH4] ⁺ [M+Fe+3- 2H] ⁺ dm/z=53.89542

1019

1020

1021

Supplementary Table 1 Summary of the Ion Identity Molecular Network (IIMN) edges, including Fe adducts. Edges connect metabolites (nodes) with iron adducts detected in the native metabolomics experiments in which iron salts were added to the system (Fig. 2F), including the siderophores rhodotorulic acid and desferrioxamine G.

1022

1023

Strain	Total Read Pairs (M)	Total Bases (Gbp)	Total Read Pairs (Trimmed) (M)	Total Bases (Trimmed) (Gbp)	Assembled Size (Mbp)	Num Contigs	N50 (kb)	GTDB Assignment
wgs_S12036Nr1	8.08	2.41	7.94	2.37	6.69	637	105.7	s_Pseudomonas_E_koreensis_A
wgs_S12036Nr2	8.10	2.42	7.96	2.37	5.92	229	247.5	s_Pseudomonas_E_siliginis
wgs_S12036Nr3	8.10	2.41	7.95	2.37	5.94	281	112.3	s_Pseudomonas_E_moraviensis_A
wgs_S12036Nr4	8.07	2.41	7.92	2.37	5.97	244	174.2	s_Pseudomonas_E_moraviensis_A
wgs_S12036Nr5	8.08	2.41	7.93	2.38	6.04	207	249.7	s_Pseudomonas_E_koreensis_F
wgs_S12036Nr6	8.12	2.42	7.98	2.38	5.92	249	233.7	s_Pseudomonas_E_siliginis
wgs_S12036Nr7	8.11	2.42	8.03	2.39	6.03	228	101.7	s_Pseudomonas_E_viridiflava
wgs_S12036Nr8	8.04	2.41	7.90	2.36	5.92	241	225.9	s_Pseudomonas_E_siliginis
wgs_S12036Nr9	8.10	2.42	7.93	2.37	6.03	289	305.8	s_Pseudomonas_E_moraviensis_A
wgs_S12036Nr10	8.11	2.42	7.97	2.38	6.05	249	196.2	s_Pseudomonas_E_moraviensis_A
wgs_S12036Nr11	8.11	2.42	7.96	2.38	5.97	282	154.9	s_Pseudomonas_E_moraviensis_A
wgs_S12036Nr12	8.06	2.41	7.92	2.36	5.97	278	164.4	s_Pseudomonas_E_moraviensis_A

1024 **Supplementary Table 2:** Whole-genome sequencing of commensal strains used in this study, with associated metadata including
1025 isolation site and genome features.

1026