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RESEARCH ARTICLE

Root exudates mediate plant defense against foliar pathogens by recruiting beneficial microbes

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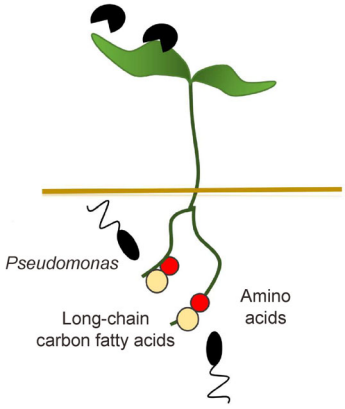
HIGHLIGHTS

- Long-chain fatty acids and amino acids application could form foliar disease resistant-soil microbial community.
- Population of *Pseudomonas* was enriched by long-chain fatty acids and amino acids application.
- The enriched *Pseudomonas* could help plant resistant foliar pathogens.

GRAPHICAL ABSTRACT



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ABSTRACT

Plants are capable of releasing specific root exudates to recruit beneficial rhizosphere microbes upon foliar pathogen invasion attack, including long-chain fatty acids, amino acids, short-chain organic acids and sugars. Although long-chain fatty acids and amino acids application have been linked to soil legacy effects that improve future plant performance in the presence of the pathogen, the precise mechanisms involved are to a large extent still unknown. Here, we conditioned soils with long-chain fatty acids and amino acids application (L + A) or short-chain organic acids and sugars (S + S) to examine the direct role of such exudates on soil microbiome structure and function. The L + A treatment recruited higher abundances of Proteobacteria which were further identified as members of the genera *Sphingomonas*, *Pseudomonas*, *Roseiflexus,* and *Flavitalea*. We then isolated the enriched bacterial strains from these groups, identifying ten *Pseudomonas* strains that were able to help host plant to resist foliar pathogen infection. Further investigation showed that the L + A treatment resulted in growth promotion of these *Pseudomonas* strains. Collectively, our data suggest that long-chain fatty acids and amino acids stimulated by foliar pathogen infection can recruit specific *Pseudomonas* populations that can help protect the host plant or future plant generations.

1 Introduction

Plants can impact their associated microbiome as an adaptation strategy when confronted by biotic and abiotic challenges (Toju et al., 2018; Ravanbakhsh et al., 2019). For instance, root exudates can influence the composition and

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function of soil microbial communities (Orwin et al., 2010; Laughlin, 2011; Vries et al., 2012; Grigulis et al., 2013), a phenomenon that is modulated by the quality and/or quantity of available soil nutrients (Bardgett and Wardle 2010). Root exudates can facilitate plant–plant, plant–insect and plant–microbe interactions (Kessler and Baldwin, 2001; Van Poecke et al., 2001; Kessler and Baldwin, 2002; Rasmann et al., 2005). For example, root exudates and root-derived compounds such as malic acid can recruit specific *Bacillus* strains to the rhizosphere (Rudrappa et al., 2008); Badri et al. (2009) showed that *Arabidopsis* ABC transporter mutant (abcg30) which releases more phenolics and there were increased abundance of PGPR or bacteria involved in heavy metal remediation was observed in rhizosphere of *Arabidopsis* compared to wild type Col-0 plants; recent studies show that *A. thaliana* produces a range of specialized triterpenes that direct the assembly and maintenance of an *A. thaliana*–specific microbiota, enabling it to shape and tailor the microbial community within and around its roots to its own purposes (Huang et al., 2019b).

Interactions between the plant and the rhizosphere soil microbiome in the context of pathogen invasion are generally more specific, with recruitment of specialized microbes potentially antagonistic to a certain pathogen. For example, wheat has been shown to recruit specific *Pseudomonas* species that produce antimicrobial compounds when confronted with "take-all" disease (Weller et al., 2002). While, beneficial rhizosphere microbes are also able to suppress pathogenesis through the induction of plant induced systemic resistance (ISR), even when the infection did not occur in the rhizosphere. The recruitment of beneficial rhizosphere microorganisms appears to associated with the modification of plant exudation patterns in response to exposure to aboveground pathogens (Yuan et al., 2018). Specifically, long-chain fatty acids and amino acids were identified to play important roles in the recruitment of potentially beneficial microbes (Yuan et al., 2018). However, which groups of the beneficial microbe are and how these compounds recruit them have not yet been elucidated.

In this study, we sought to identify the key microbial taxa enriched in soils amended with rhizosphere cocktails that mimic those produced by plants upon foliar pathogen infection. Furthermore, key bacterial groups were isolated and assayed for their ability to induce foliar pathogen resistance. By combining these approaches, we linked specific exudates classes to the recruitment of bacterial taxa groups that contribute to plant resistance against aboveground pathogen attack.

2 Materials and methods

2.1 Impacts of root exudate classes on the soil microbiome

To examine the effect of key exudate compounds on the soil microbiome, we selected four categories of different secreted exudates based on our previous results (Yuan et al., 2018). They are long-chain fatty acids (LCFA) (pentadecanoic acid, hexadecanoic acid, palmitoleic acid, octadecanoic acid, and arachidic acid), amino acids (AA) (isoleucine, leucine, methionine, proline, tryptophan, and ornithine), short-chain organic acids (SCOA) (citric acid, aconitic acid, succinic acid, and malic acid) and sugars (maltose, ribose, glucose, sucrose, fructose, and xylose). In our previous study, LCFA and AA application to soil can increase the plant resistance to foliar pathogen growing in that soil. While this beneficial effect was not seen when applied with SCOA and sugar. The soil conditioning process was as previously described (Yuan et al., 2018). Briefly, for the AA, SCOA, and sugar category, watery solutions were prepared containing each of the selected compounds in equal dosage and to a final total concentration of 10 mM. For LCOAs, the total concentration was 10 μM due to their lower solubility. The 6-well plates with 15 g soil in each well were pre-incubated in a growth chamber at 30°C for 1 week. Each well then received 1.5 mL of L + A solution or S + S solution or water twice a week for 8.5 weeks. The L + A solution contains equal volumes of the LCOA and AA solutions, and the S + S solution contains equal volumes of SCOA and sugar solutions. Each treatment consisted of 3 plates with 18 replicates. All plates were randomly placed during the incubation period.

2.2 DNA extraction PCR amplification and sequencing

The soil from two wells are randomly pool as one sample, and total soil DNA was extracted from all 27 samples. Extractions were carried out on 500 mg of soil (wet weight) using the Power Soil DNA Isolation kit (Mo Bio Laboratories Inc., Carlsbad, CA, USA), according to the manufacturer's instructions. DNA was extracted from two technical replicates per sample to minimize DNA extraction bias. Samples were stored at −20°C, and technical replicates were pooled before performing polymerase chain reaction. DNA quality was assessed according to the 260/280 nm and 260/230 nm absorbance ratios using a NanoDrop ND-2000 spectrophotometer (NanoDrop, ND2000, Thermo Scientific, 111 Wilmington, DE). Bacterial 16S rRNA gene fragments were amplified from the extracted DNA using primers 341F (CCTAYGGGRBGCASCAG) and 806R (GGACTACHVGGGTWTCTAAT) following the PCR conditions previously described (Yuan et al., 2018). Amplicons were subjected to 250-bp paired-end sequencing on the Illumina MiSeq sequencing platform at the Genomics Core of Michigan State University.

2.3 Amplicon sequence processing and analysis

Amplicon sequences were analyzed using the "DADA2" package in R environment (version 3.6.1). Initial sequence quality was assessed using the "plotQualityProfile" function. Due to low sequence quality at the 3′-ends of the reads, joining paired-ends resulted in unreliable reads reducing the number of paired-end reads that passed quality control. We therefore only used the forward reads containing the relatively

short but highly discriminating V3 region (Callahan et al., 2016). Sequences were truncated at base 120 and trimmed until base 15. This resulted in relatively short reads (~120 bp) of high quality from which actual sequence variants (ASVs) were identified.

The DADA2 pipeline produced an ASV count table containing 240 000 usable reads and ~2350 ASVs. Some of them were judged to likely be from chimeric sequences that were not filtered out in the DADA2 pipeline (as a consequence of using only the forward reads) and were thus excluded from further analysis. The resulting final ASV table contained ~200 000 high-quality reads belonging to ~2000 ASVs. For taxonomy assignment of ASVs, we referenced the protocol of the Earth Microbiome Project (Thompson et al., 2017). Taxonomic assignment of ASVs was accomplished with the assign taxonomy function against the SILVA 16S rRNA database.

2.4 Selection of bacterial isolates

The 0.5 g soil of each treatment was stored in 1 mL of 5 mM $MgSO_4$ with 25% glycerol (v/v) at −80°C at the end of the conditioning experiment for further isolation of beneficial microbes. Before isolation, six replicates of soil samples treated with L + A were thawed at room temperature and suspended by vertexing for 120 s. For the isolation of culturable bacterial species, a serial dilution of each soil sample was plated on 1/10 tryptic soy agar (TSA; Difco) (Rocelle et al., 1995) amended with 200 mg L^{-1} Delvocid (DSM; active compound: natamycin) to prevent fungal growth and plates were incubated for 3–5 days at 28°C. Then, we selected as many as different strains according to morphological characteristics and size of colony, and about ~20 strains were picked out from each plant. A total of 200 bacterial colonies with unique morphologies were selected and streaked on tryptic soy agar (TSA).

Single colonies from pure cultures were inoculated in 1/10 strength tryptic soy broth (TSB), incubated overnight at 28°C at 180 r min^{-1}, and stored at −80°C in 25% (v/v) glycerol.

2.5 Isolate identification

A loop of bacterial cells was added to 20 µL of water, incubated for 15 min at 95°C and immediately cooled on ice. This bacterial lysate was diluted ten times with water and cell debris was removed by centrifugation (1 min, 10 000 × *g*). We adopted a one-step barcoded PCR protocol (Goodman et al., 2011), in combination with Illumina sequencing to identify the V5–V7 sequences of bacterial 16S rRNA genes of all isolates. Isolates in 96-well microtiter plates were indexed using the degenerate primers 799F/1193R containing barcodes to amplify the variable regions V5–V7. For PCR amplification, DNA from 1.5 µL of lysed cells was amplified using 2×Premix Taq (Takara Biotechnology, Dalian Co. Ltd., China), 1 µL of 0.2 µM forward primer with an 18-bp linker sequence and 1 µL of 0.2 µM reverse primer (1193R) in a 20 µL volume. PCR amplification was performed under the following conditions: 95°C for 2 min, followed by 40 cycles of 95°C for 30 s, 58°C for 30 s and 72°C for 30 s, and a final elongation step at 72°C for 10 min. PCR products of each 96-well microtiter plate were combined and subsequently purified. Sequences were quality filtered and demultiplexed. OTUs were clustered at 100% similarity with UNOISE3, which has a built-in error correction to reduce the influence of sequencing errors (Edgar, 2016).

For further identification, the 16S rRNA gene of each isolate was amplified with primers F27/R1492 (Höfle et al., 2005). Two microliters of the colony lysate were added to a total volume of 50 µL PCR mixture (5 µL 10 × Dreamtaq buffer (Thermo Sci-entific), 1 µL 10 mM dNTP's, 2.5 µL 10 µM forward primer F27, 2.5 µL 10 µM reverse primer R1492, 1 µL Dreamtaq polymerase (Thermo Scientific), 36 µL H_2O. PCR conditions were 5 min at 94°C, followed by 30 cycles of 1 min at 94°C, 1 min at 55°C and 1 min at 72°C, and a final extension of 10 min at 72°C. PCR products were examined by electrophoresis on 1.5% agarose gels in 1×TAE buffer (40 mMTris-acetate/1 mM EDTA, pH8) and sequenced by GenScript (Nanjing). Isolate taxonomy was determined through the Sequence Match function of the ribosomal database project (RDP) (Quast et al., 2013). For further exploration of the phylogenetic relationship between enriched ASVs from high throughput sequencing and the isolates, the similarity was showed by alignment the sequence of ASVs to isolates.

2.6 Microbial induced foliar pathogen resistance assay

For this assay, 7-day-old *Arabidopsis* seedlings (Col-0) were prepared and then transplanted into an autoclaved mixture of vermiculite and sand (volume:volume = 1:1) in 6-well plates, as described previously (Yuan et al., 2018). One week after transplantation, the isolates were pre-cultured in LB broth and applied after re-suspension with autoclaved water at a final concentration of 5×10^8 per well. Autoclaved water was added as the negative control. Then, each tested strain was added to 6 transplanted plants as one replicate and 6 replicates were performed. The test soils were collected from a long-term field experiment that began in January 2014 in the Hengxi town of Nanjing, Jiangsu Province (32°02′N, 118°50′E). For each pot, 5 mL of MS medium were added to supply plants with the necessary nutrition during plant growth period. After one week, *Pseudomonas syringae* pathovar *tomato* DC3000 (hereafter referred to as *Pst*) strain was inoculated onto the *Arabidopsis* leaves as previously described (Yuan et al., 2018). Disease incidence was determined 7 days after infection. Isolates that reduced disease incidence were recognized as beneficial microbes.

2.7 Growth promotion effects of distinct exudate compounds on isolated beneficial strains

Flask culturing was used to test the influence of specific exudate compounds on the growth of the bacterial isolates. Isolates were inoculated in 1/10 strength TSB media, shaken at 170 r min^{-1} for 24 h, and cell density adjusted by the

addition of sterilized water with the final OD_{600} reached 0.6. Then, 1.5 mL fresh bacterial suspensions (1%) were inoculated into 250-mL conical flasks with 150 mL 1/10 strength TSB liquid media, and 1.5 mL of L + A or S + S solution were added to liquid media with the same concentration as used in soil condition experiment. Controls contained sterile water and same volume of the bacterial suspensions. Cultures were shaken at 170 r min^{-1} and the absorbance at OD_{600} was used to determine bacterial growth curves.

2.8 Chemotaxis effects of distinct exudate compounds on isolated beneficial microbes

The modified capillary assay was performed based on the method described by Adler et al. (1973) and our previous publication (Yuan et al., 2015) to quantitatively determine the chemotaxis response of the isolates to the distinct exudate compounds. The strain was grown in LB media until an OD_{600} of 0.8 was reached. Briefly, the cells were resuspended in the chemotaxis buffer [100 mM potassium phosphate (pH 7.0) with 20 μM EDTA] at concentration of OD_{600} = 0.8. A Petri dish 60 mm in diameter was filled with 10 mL of the cell suspension prepared above. Standard 1 μL capillaries loaded with the exudate cocktails L + A or S + S solution with the same concentration as mentioned above were immersed in the cell suspension in Petri dishes, while the chemotaxis buffer was performed as the negative control. After 30 min incubation in sterile bench, the suspension was then diluted and plated on TSB plates. The CFU were determined by plating on TSB plates and incubating at 37°C for 24 h. Each treatment was replicated three times.

2.9 Statistical methods

Statistically significant differences ($p < 0.05$) among treatments were evaluated by Student's *t*-test or ANOVA using SPSS. Differences in bacterial community composition across treatments were tested using PERMANOVA (Adonis, transformed data by Bray–Curtis, permutation = 999), implemented in R version 3.4.3. The DESeq function of the"DESeq2" package (version 1.18.1) (Love et al., 2014) was employed to test for differentially abundant ASVs among conditioned soil samples. Statistical significance was based on *p*-value < 0.05 (with FDR < 5% under the Benjamini-Hochberg correction). Statistical analyses of the community of 16S rRNA gene sequencing data were performed using the phyloseq package in the R environment (version 3.6.1). β-diversity (PCoA based on Bray–Curtis dissimilarities) was calculated using the "phyloseq" package (version 1.22.3). Statistical significance of the β-diversity between treatments was calculated through analysis of similarity (adonis), as implemented in the R environment. The DESeq function of the "DESeq2" package (version 1.18.1) was employed to test for differentially abundant ASVs. Statistical significance was based on *p*-value < 0.05 (with FDR < 5% under the Benjamini-Hochberg correction). Graphs of the microbiome data were created using the "ggplot2" package (version 2.2.1).

3 Results

3.1 Impact of exudation cocktails on soil bacterial communities

We analyzed the composition of bacterial communities after soil was exposed to two types of exudation cocktails using 16S rRNA gene amplicon sequencing. Average read count per sample was 47 705±8149. Bacterial communities were highly diverse with the number of ASVs ranging from 480 to 642 per sample in L + A soils (596±62), between 303 and 487 per sample in S + S soils (399±69), with between 313 and 589 per sample in the water/control soils (461±76). Over all of the groups, the majority of ASVs belonged to the phyla Candidatus_Saccharibacteria (29.1%), Proteobacteria (25.6%), Cyanobacteria (15.7%), Acidobacteria (6.0%), Bacteroidetes (5.6%), and Actinobacteria (7.6%). Exudate cocktails impacted the composition of the soil bacterial community (Fig. 1A). The S + S treatment significantly increased the relative abundance of Candidatus_Saccharibacteria (from 7.8% to 62.4%), while the L + A addition significantly increased the relative abundance of Proteobacteria (from 20.4% to 46.1%) relative to the control samples. Both treatments decreased the relative abundance of Cyanobacteria from 38.8% in the control samples to 3.6% and 4.6% in the L + A and S + S samples, respectively.

Principal coordinate analysis (PCoA), based on the detected ASVs, showed a significant difference (PERMANOVA, $p = 0.001$, $R = 0.28$) in community composition between the two exudates cocktails and the control samples (Fig. 1B). Amino acid and long-chain fatty acid additions increased the alpha-diversity of the soil bacterial community, while the addition of sugars and short chain organic acids decreased the bacterial diversity, as compared with the water control (Fig. 1C).

Compared to the control soils, a total of 22 ASVs were relatively enriched in the L + A samples, yet depleted in S + S samples (Fig. 1D). In order of relative abundance, eight ASVs with relative abundances > 1% belonged to the genera *Sphingomonas*, *Azohydromonas*, *Pseudomonas*, *Roseiflexus*, *Azoarcus*, *Flavitalea*, and *Burkholderia*. Previously, it was found that *Sphingomonas*, *Pseudomonas*, and *Roseiflexus* increased in the relative abundance greater in pathogen-conditioned soils (Yuan et al., 2018). In total, L + A exudates influenced general bacterial community composition, while also recruiting a select range of specific bacterial taxa.

3.2 Microbial isolates and their impacts on foliar pathogen resistance

Of the 156 bacterial colonies with unique morphologies selected for further analysis, 23 were putatively identified as belonging to the genera *Sphingomonas* and *Pseudomonas*, according to phylogenetic analysis (Fig. 2A). Given the previous link between these general and disease suppression (Yuan et al., 2018), we further examined the

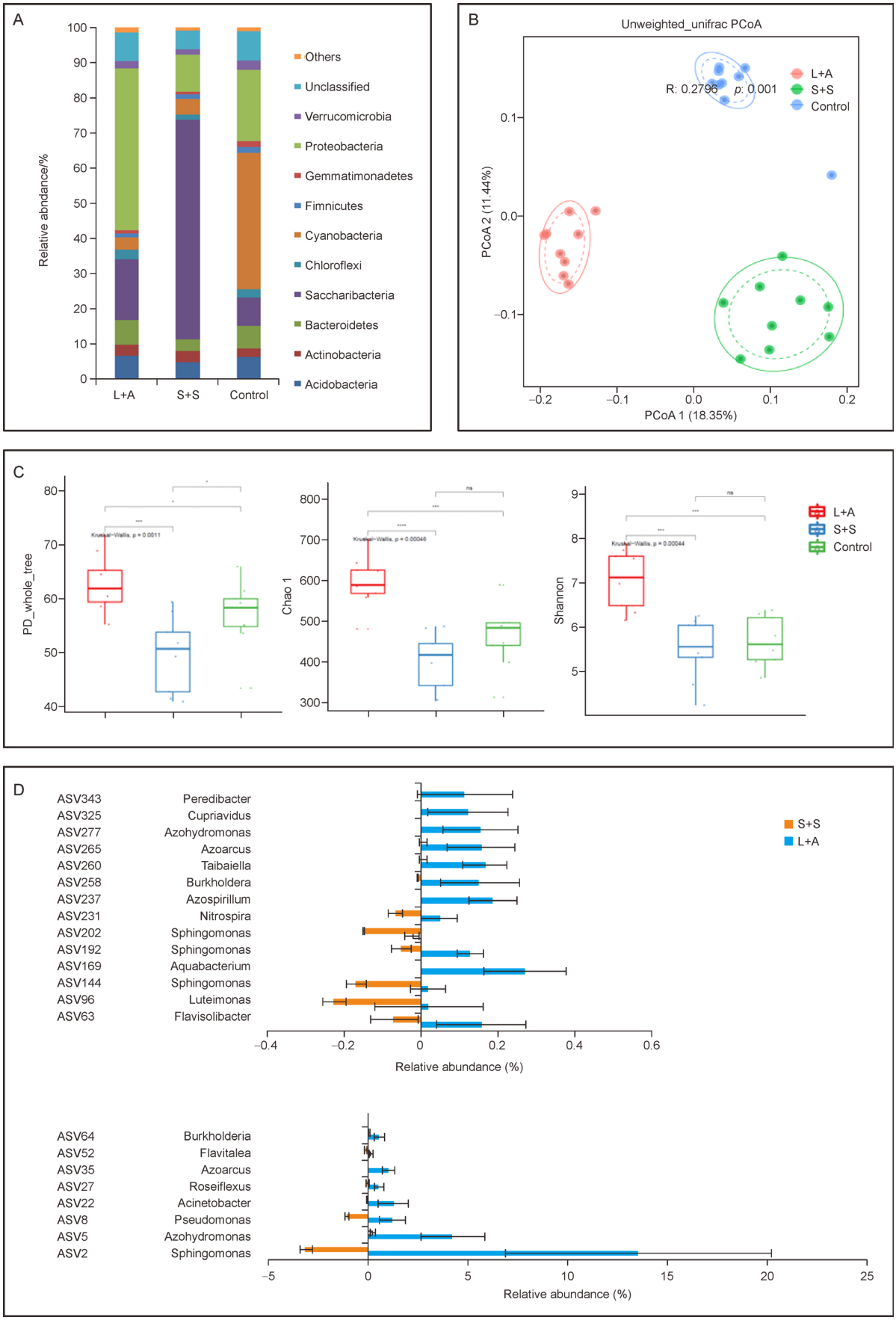

Fig. 1 (A) Relative abundance (%) of the major bacterial phyla with significant differences present in the microbial communities of control and exudate-conditioned soil samples. (B) Principal coordinates analysis (PCoA) with Bray–Curtis dissimilarity of the rhizosphere bacterial community in exudates or water conditioned soil. (C) The *alpha* indices (PD_whole_tree, Chao 1, and Shannon) of rhizosphere bacterial community in each sample. Asterisk indicated the significant difference between two groups. The relative abundance of 22 ASVs that were enriched in the L + A samples, yet relatively depleted in the S + S samples, as compared to the controls.

ability of these 23 isolates to induce foliar pathogen resistance against foliar infection when applied to plants in sterilized materials. Of these, ten isolates showed an ability to decrease the incidence of disease in our *Arabidopsis* assay (Fig. 2B). These ten bacteria were all identified as *Pseudomonas* sp., including *Pseudomonas nitroreducens*, *Pseudomonas plecoglossicida*, and *Pseudomonas putida* with high average nucleotide identities (>99%) (Fig. 2C).

3.3 Effects of exudate cocktails on isolate growth

To further investigate whether the strains with ability to induce foliar pathogen resistance were impacted by the application of specific exudates groups, we examined the ability of exudate application to stimulate growth of the ten strains identified above. The growth of strains P14 and P90 was promoted by L + A yet inhibited by S + S (Fig. 3A). Strains P89 and P137 grew better than the control in response to both L + A and S + S (Fig. 3A). Strain P30 was only promoted by L + A, while strain P103 was inhibited by both L + A and S + S (Fig. 3A). The other four strains (P29, P17, P69, and P34) were not significantly influenced by the exudate's treatments (Fig. 3A).

3.4 Chemotaxis effects of exudate cocktails on the tested isolates

We then tested if the compounds could recruit the isolates. Results indicated that L + A could significantly attract strain P14, P30, P103, and P34, while S + S could only attract P103 and P34 (Fig. 3B). It is also found that the L + A showed higher

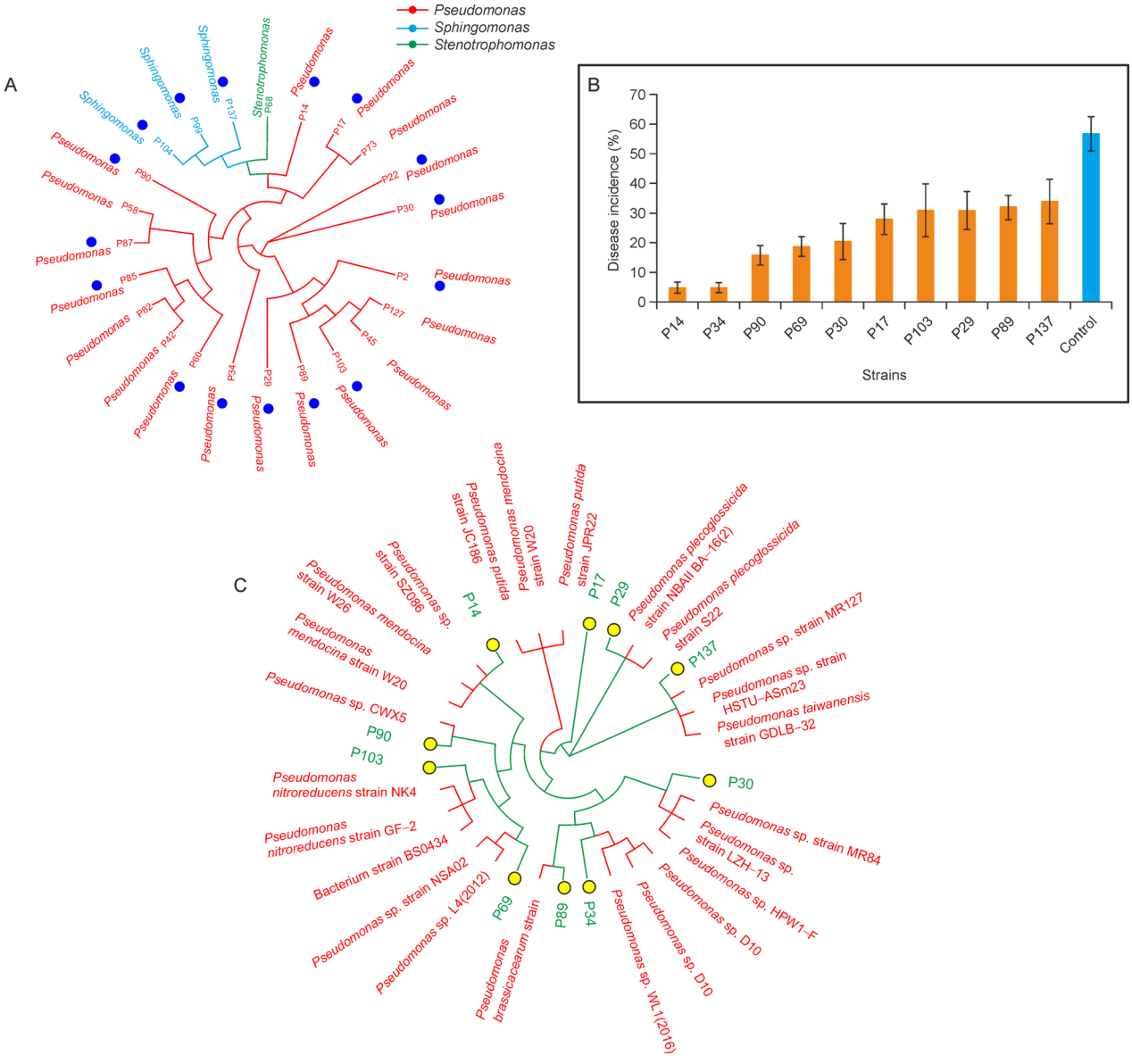

Fig. 2 (A) Maximum-likelihood phylogenetic tree of all 23 representative OTUs clustered with preliminary identification using the high-throughput sequencing method. (B) Foliar pathogen resistance inducement effects of the selected isolates. (C) Phylogenetic tree of the ten beneficial microbe identified as *Pseudomonas*.

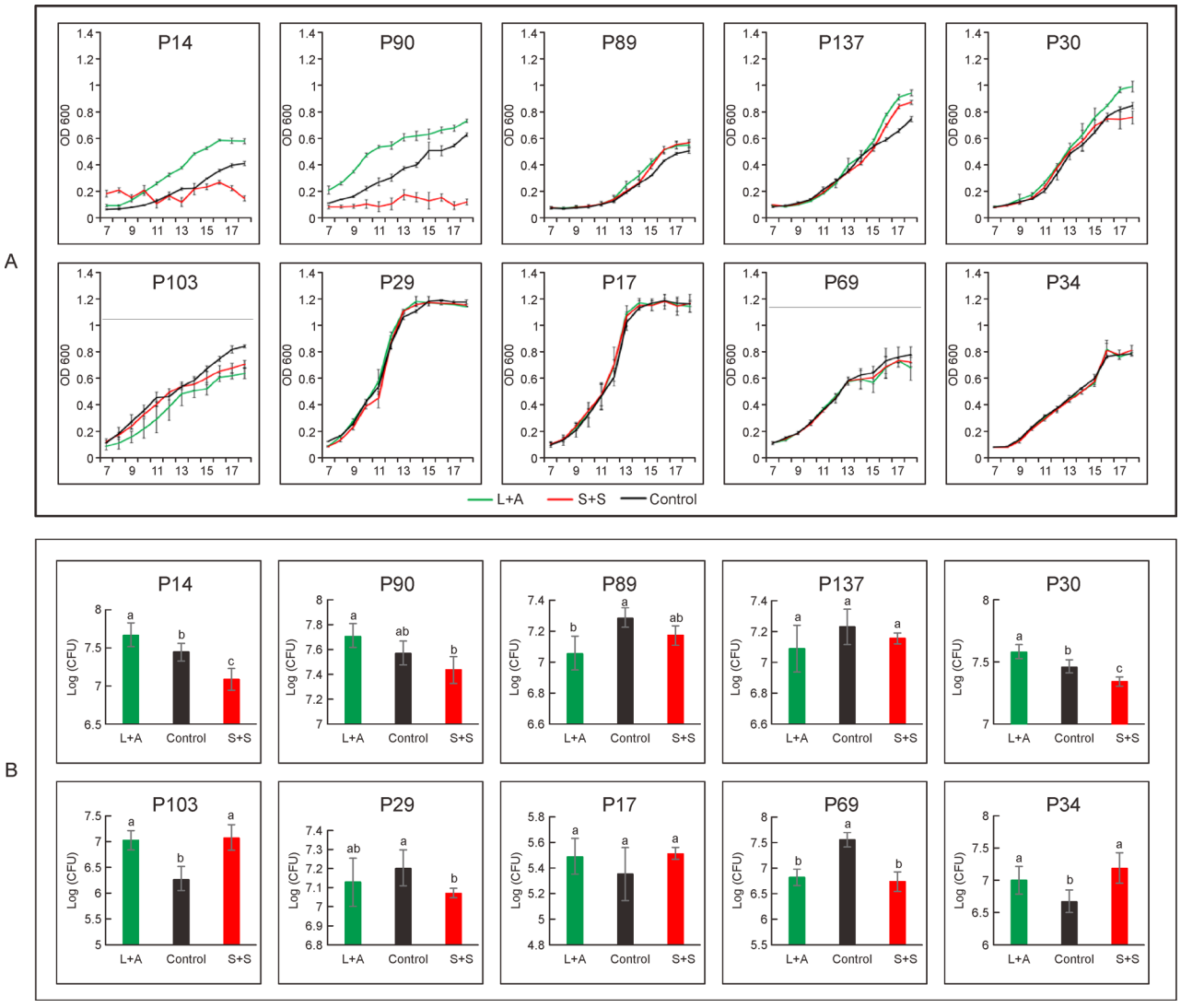

Fig. 3 (A) Effects of two types of exudate cocktails on the growth of 10 tested isolates. (B) Chemotaxis effects of two types of exudate cocktails on the 10 tested isolates Bars indicate the standard errors of the means from three replicates. Columns with different letters are statistically different (ANOVA $p < 0.05$) among each group.

attraction for strain P90 than S + S (Fig. 3B). Both two types of exudates could not attract strain P89 and P69. Compared with S + S, L + A showed more stronger recruitment effect on the isolates, while S + S showed repellent effect on some isolates such as P14, P90, P30, P29, and P69 (Fig. 3B).

4 Discussion

We previously found that foliar pathogen (*Pst*) infection induced the root secretion of long-chain fatty acids and amino acids, and further found that application of these two groups of compounds could promote host plant systemic resistance, presumably by helping to recruit beneficial microbes (Yuan et al., 2018). Here, we show that addition of long-chain fatty acids and amino acids significantly altered the composition of soil bacterial community by increasing the proportion of Proteobacteria. Conversely, sugars and short-chain organic acids increased the proportion of Candidatus_-Saccharibacteria (Fig. 1). We also found that the relative proportion of *Sphingomonas*, *Pseudomonas*, *Roseiflexus,* and *Flavitalea* increased upon the application of long-chain fatty acids and amino acids, yet decreased in response to application of sugars and short-chain organic acids. These results are in line with our previous data that found higher relative abundances of some of these groups in treatments with foliar pathogen infection (Yuan et al., 2018).

Of foliar pathogen resistance-inducing taxa identified in our study, *Pseudomonas* has been extensively reported to the ability to induce ISR in a range of plant species (Weller et al., 2007; Pieterse et al., 2014). This genus and *Sphingomonas*, a bacterial genus closely affiliated with *Pseudomonas*, were previously reported to be enhanced by the application of long-chain fatty acids and amino acids (Kim et al., 2000). After preliminarily identification using high-throughput sequencing

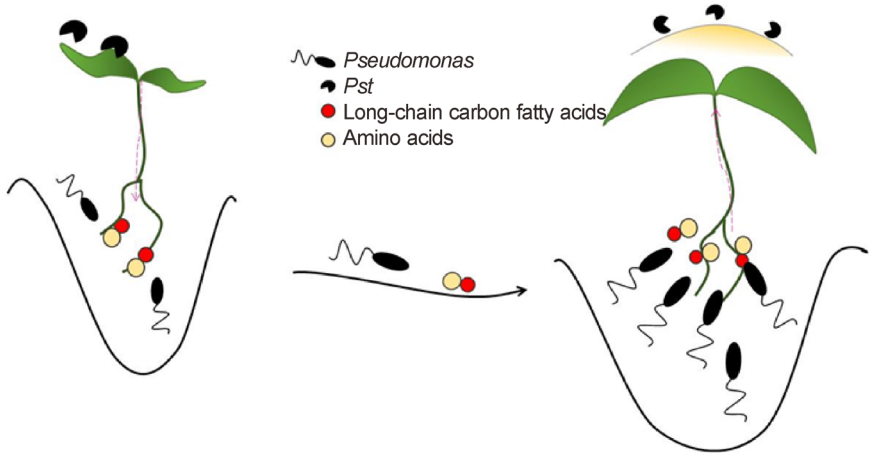

Fig. 4 Mechanistic model showing the plant release of long-chain fatty acids and amino acids to recruit *Pseudomonas* as a response to foliar pathogens.

with a portion of the 16S rRNA gene, *Sphingomonas* and *Pseudomonas* were further studied in the foliar pathogen resistance inducement assay. Ten strains, confirmed to induce foliar pathogen resistance of host plant, were all classified to *Pseudomonas* (Table 1) and showed 100% similarity with the ASV8 (*Pseudomonas*), and only about 80% similarity with ASV2 (*Sphingomonas*). This is supported by previous results that host plants can recruit *Pseudomonas* to their rhizosphere when faced with foliar pathogen attack (Haney et al., 2018). Here, we also make the link between induction of specific classes of exudates to the recruitment of specific taxa capable of inducing foliar pathogen resistance.

It has been found that distinct root exudate compounds influence the cultivation of specific soil microbes. For instance, recent results from Huang et al. (2019a) has reinforced this by demonstrating that terpenes from roots served to cultivate a highly distinct microbial community. Similarly, long-chain fatty acids and amino acids that are over produced under foliar pathogen infection can attract and then promote the growth of some *Pseudomonas* strains (Fig. 2). Conversely, sugars and short-chain organic acids were shown to exhibit poor beneficial microbe growth promotion and recruitment effects. It should be noted that our study was only able to examine a modest range of strains in this regard, and as such, further research is necessary to determine the full mechanisms underlying the recruitment and growth promotion of this important plant-growth promoting bacterial group.

Table 1 Sequencing alignment of ASV2 and ASV8 to all ten isolates.

Isolates	Similarity[a] (ASV2)	Similarity[a] (ASV8)
P14	100%	82%
P34	100%	82%
P90	100%	85%
P69	100%	84%
P30	100%	82%
P17	100%	81%
P103	100%	80%
P29	100%	80%
P89	100%	83%
P137	100%	86%

Note: “a” represented the base matching degree for sequence of ASV2 or ASV8 aligned to isolates.

5 Conclusion

In this study, several strains classified as *Pseudomonas* were identified as beneficial microbe with foliar pathogen resistant inducement and were stimulated by the application of long-chain fatty acids and amino acids. These results allow us to complete the whole plant–soil feedback model, which has partly been presented in our previous work (Yuan et al., 2018). Upon foliar pathogen infection, plants release more long-chain fatty acids and amino acids to their rhizosphere to recruit and stimulate specific *Pseudomonas* population. These *Pseudomonas* populations are then able to induce systemic resistance affording protection from foliar pathogen attack (Fig. 4).

Competing interests

The authors declare that they have no competing interests.

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Availability of data and materials

Sequence data are deposited in the NCBI Sequence Read Archive (SRA) database (SRP243322).

Authors' contributions

JY: performed all experiments; JY and QS designed the study, and wrote the majority of the manuscript; JY, TW and MZ: analyzed the data; GAK: participated in the design of the study, provided comments and edited the manuscript.

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