

<https://doi.org/10.1038/s42003-024-07208-z>

# Cross-niche protection of kiwi plant against above-ground canker disease by beneficial rhizosphere *Flavobacterium*

Wei Zheng<sup>1,2,6</sup>, Nana Wang<sup>1,3,6</sup>, Guoliang Qian<sup>4</sup>, Xun Qian<sup>5</sup>, Wei Liu<sup>1,2</sup> & Lili Huang<sup>1,2</sup>

Beneficial rhizosphere microorganisms are widely employed to shield crops from underground pathogen infections. In this study, we challenge this conventional idea by employing rhizosphere soil bacteria to safeguard kiwi plants against the above-ground canker, caused by *Pseudomonas syringae* pv. *actinidiae* (Psa). Microbiome comparisons were conducted in different resistant cultivars *Actinidia chinensis* var. *deliciosa* ‘Hayward’ and *A. chinensis* var. *chinensis* ‘Hongyang’. Our findings reveal the most notable disparity in the rhizosphere soil microbiome, with the *Flavobacterium* significantly enriched in the rhizosphere soil of more resistant cultivar, ‘Hayward’. We isolated *Flavobacterium* isolates and observed their efficacy in preventing Psa infection, which is further confirmed in field trial by using a representative strain *Flavobacterium soyae* F55. Furthermore, undescribed gene clusters responsible for antimicrobial metabolite biosynthesis were identified in *F. soyae* F55, and *F. soyae* F55 growth was evidently promoted by the root exudates of ‘Hayward’. The results underscore the potential of beneficial rhizosphere soil bacteria in protection against above-ground disease.

The microbiome is closely related to our lives. For example, the intestinal microbes in humans (and insects) can play an important role in intestinal physiology and health by influencing intestinal permeability, digestion, metabolism and immune response<sup>1</sup>. In plants, the microbiome is considered as the plant’s second genome, or ‘holobiont’<sup>2</sup>. Plants provide a large number of compartments for microorganisms, whose members maintain beneficial, harmful or neutral relationships with plants and participate in plant growth, stress tolerance, nutrient absorption, and disease resistance<sup>3,4</sup>. Rhizosphere soil and endospheric microorganisms are research hotspots, with their microbiome composition and function extensively studied in model plants and various agricultural crops, including wheat, maize, rice, tomato, and potato<sup>5,6</sup>. Studies have shown that disease resistance in plants is associated with particular communities of beneficial microorganisms, and disease-resistant plants can enrich beneficial microbes that help them to resist microbial invasion by pathogen. Gong et al. found that there was a significant difference in the rhizosphere soil microbiome between different eggplant individuals, and resistant eggplant can reduce the occurrence of bacterial wilt through the enrichment of *Bacillus* microbe in rhizosphere soil<sup>7</sup>. Similarly, the study by Yin et al. showed that tomato resistant cultivars reduced the occurrence of bacterial wilt disease by enriching *Sphingomonas* sp. Cra20 and *Pseudomonas putida* KT2440 in the rhizosphere soil<sup>8</sup>. There

has been increasing evidence for using beneficial microbe from rhizosphere soil can shape the rhizosphere soil microbiome composition and function that inhibit soil-borne disease. However, instances about cross-niche protection of plants against above-ground disease by beneficial rhizosphere soil bacteria are poorly described.

Kiwi (*Actinidia Lindl.*) is widely recognized as nutritious food owing to high vitamin C content and abundance of other essential nutrients. Since the 1970s, kiwi has been cultivated worldwide, providing economic value to the fruit industry. China is the world’s major producer and distributor of kiwi<sup>9</sup>. However, kiwi bacterial canker (KBC), caused by *Pseudomonas syringae* pv. *actinidiae* (Psa), has seriously harmed the development of the kiwi industry and even caused the destruction of orchards<sup>10,11</sup>. Disease management strategies for KBC currently primarily rely on copper (Cu)-based agents and antibiotics<sup>12</sup>. KBC mainly impact the above-ground of kiwi plant, typical field symptoms of KBC include polygonal brown necrotic spots with yellow halos on leaves, brown discoloration and necrosis of flower buds, cankers with exudates on canes and trunks<sup>13</sup>. KBC also colonize the root but nearly no symptom. Thus, the rhizosphere soil microbiome of different resistant kiwi cultivars potentially acts as an excellent source to discover beneficial rhizosphere soil microbes that achieves cross-niche protection of plants against the above-ground canker disease.

<sup>1</sup>State Key Laboratory of Crop Stress Biology for Arid Areas, Yangling, Shaanxi, China. <sup>2</sup>College of Plant Protection, Northwest A&F University, Yangling, Shaanxi, China. <sup>3</sup>College of Life Science, Northwest A&F University, Yangling, Shaanxi, China. <sup>4</sup>College of Plant Protection, Nanjing Agricultural University, Weigang, Nanjing, Jiangsu, China. <sup>5</sup>College of Natural Resources and Environment, Northwest A&F University, Yangling, Shaanxi, China. <sup>6</sup>These authors contributed equally: Wei Zheng, Nana Wang. ✉ e-mail: [huanglili@nwsuaf.edu.cn](mailto:huanglili@nwsuaf.edu.cn)

The present study presents a case study on how to employ comparative microbiome investigations to discover beneficial rhizosphere soil bacteria in kiwi plants for controlling KBC in a cross-niche manner. Specifically, this research discovered and examined how *Flavobacterium* isolates enriched in the rhizosphere soil of *Psa*-resistant kiwi cultivar can defend against the pathogen *Psa*, which primarily attacks from above-ground. Harnessing the beneficial effects of these microbiome bacteria, represented by *Flavobacterium*, may offer potential strategies to enhance kiwi crop health and resilience in agricultural settings.

## Results

### Rhizosphere soil microbiota structure differed between 'Hongyang' and 'Hayward'

Amplicon sequencing was performed on endophytic bacteria within distinct compartments and rhizosphere soil microorganisms from both 'Hongyang' and 'Hayward' cultivars. Following quality trimming and the allocation of reads to specific samples, the 16S rRNA gene dataset retained 41,828–69,621 high-quality reads, averaging 373 bp in length (Supplementary Table 1). To standardize the sampling effort across plant samples, each sample was rarefied to 34,362 sequences before calculating diversity indices. A total of 4260 OTUs were identified from the bacterial community. Our findings revealed no significant differences in alpha diversity within the root, leaf, and cane endophytic compartments between 'Hongyang' and 'Hayward'. However, notable distinctions were observed in the rhizosphere soils of 'Hongyang' and 'Hayward' ( $P < 0.05$ ) (Fig. 1a, b). The observed species index indicated the highest bacterial diversity in rhizosphere soil samples, with reduced diversity estimates in the remaining three compartments (Fig. 1a). Consistent results were observed for the shannon index estimates (Fig. 1b). We assessed bacterial community structures' disparities between 'Hongyang' and 'Hayward' in different compartments using Principal Component Analysis (PCA) at the Operational Taxonomic Unit (OTU) level. At the OTU level, PC1 explained 31.6%, and PC2 explained 13.6% of the total variation. Rhizosphere soil exhibited significant differences between 'Hongyang' and 'Hayward' (PERMANOVA,  $P = 0.0013$ ). However, no significant differences were observed in the root, leaf, and cane endophytic compartments between 'Hongyang' and 'Hayward' (Fig. 1c and Supplementary Table 2).

### *Flavobacterium* significantly enriched in the rhizosphere soil of 'Hayward' compare to 'Hongyang'

To investigate the bacterial community composition in different compartments of 'Hongyang' and 'Hayward', we assigned Operational Taxonomic Units (OTUs) to the genus level. At this level, 3015 OTUs (33.6%) were annotated, identifying 451 genera. The highly relatively abundant *Unidentified\_Rickettsiales* (79.3%–98.8%) were observed in leaf and cane

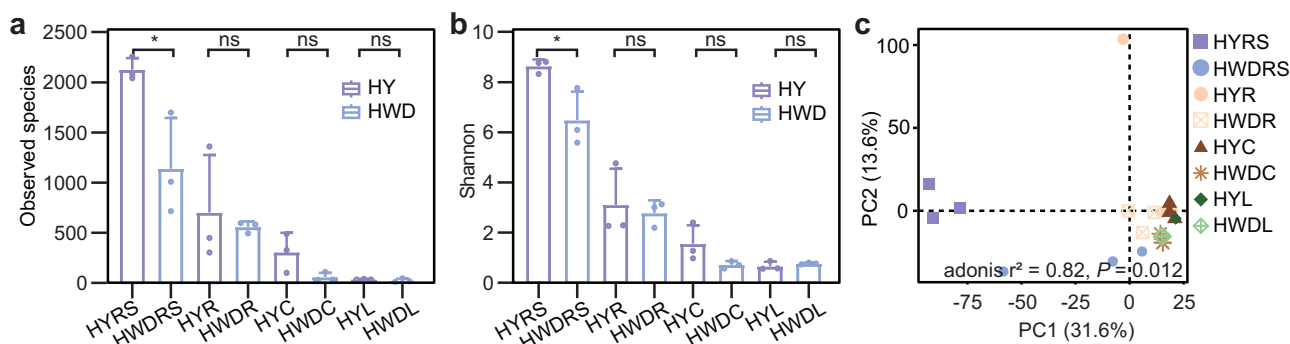
endophytic compartments (Fig. 2a). In the root endophytic compartments, *Unidentified\_Enterobacteriaceae* (46.4% in 'Hongyang' and 15.9% in 'Hayward'), *Unidentified\_Rickettsiales* (12.7% in 'Hongyang' and 37.3% in 'Hayward'), *Bacillus* (7.8% in 'Hongyang' and 10.8% in 'Hayward'), and *Streptomyces* (1.1% in 'Hongyang' and 9.3% in 'Hayward') showed high relative abundance (Fig. 2a). In the rhizosphere soil, *Flavobacterium* (5.9% in 'Hongyang' and 16.7% in 'Hayward'), *Terrimonas* (2.1% in 'Hongyang' and 18.2% in 'Hayward'), *Lacibacter* (0.4% in 'Hongyang' and 5.9% in 'Hayward'), and *Steroidobacter* (2.5% in 'Hongyang' and 2.0% in 'Hayward') were identified (Fig. 2a).

We conducted a comparison of bacterial community compositions at the genus level across different compartments of 'Hongyang' and 'Hayward' using both Student's *t*-test and LEfSe analysis. Our findings revealed significant differences in the rhizosphere soils of 'Hongyang' and 'Hayward', with 35 genera exhibiting notable distinctions according to the Student's *t*-test. Conversely, no significant differences were observed in the endophytic compartments between the two cultivars (Fig. 2a, Supplementary Fig. 1, and Supplementary Table 3). Furthermore, the Linear Discriminant Analysis Effect Size (LEfSe) analysis highlighted a significant enrichment of *Flavobacterium* in the 'Hayward' rhizosphere soil, consistent with the results of the analyses described above. *Lacibacter* and *Terrimonas* were also enriched in the rhizosphere soil of 'Hayward' (Fig. 2b). At the OTU level, *Flavobacterium* OTU6, OTU1542, OTU337, OTU43, OTU28, and OTU84 showed higher relative abundances in 'Hayward' rhizosphere soil compared to the 'Hongyang' rhizosphere soil (Fig. 2c).

### *Flavobacterium* isolates inhibited *Psa* invasion in kiwi plants

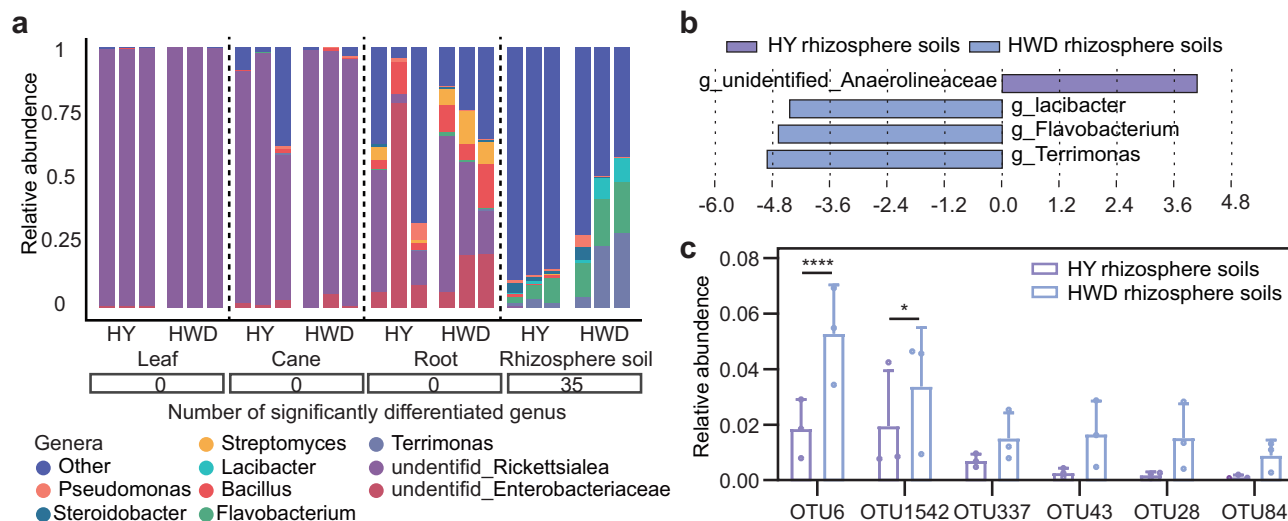
We isolated and identified *Flavobacterium* isolates from the rhizosphere soil of the 'Hayward' cultivar to investigate their potential functions. Using sequence similarity analysis, we aligned bacterial 16S rRNA gene sequences obtained from isolates with the 16S rRNA gene V3–V4 region sequences acquired through Illumina NovaSeq 6000 sequencing. A total of 35 bacterial isolates were grouped into 17 OTUs, identified in our corresponding 16S rRNA amplicon pyrosequencing data using different media (PDA, LB, TSB) (Fig. 3a). Among these isolates, four were identified as belonging to the *Flavobacterium*, specifically, *Flavobacterium* OTU1542 (F55), *Flavobacterium* OTU6, *Flavobacterium* OTU337, and *Flavobacterium* OTU193 (Fig. 3a, b).

We assessed the antagonistic effects of *Flavobacterium* F55, OTU6, OTU337, and OTU193 against *Psa*. The zone of inhibition assay revealed their antagonistic activity against *Psa*, with F55 exhibiting particularly robust effects (Fig. 3c). Additionally, we investigated the disease-suppressive activity of these isolates against *Psa* on detached leaf discs and canes of 'Hongyang' cultivar. Inoculation with *Flavobacterium* F55, OTU6, OTU337, and OTU193 provided significant protection against *Psa*



**Fig. 1 | Comparison of microbial diversity and community structure in endophytic and rhizosphere soil from two kiwi cultivars. a** Observed species diversity index. **b** Shannon diversity index. The observed species diversity index and the shannon diversity index were evaluated as alpha diversity. Asterisk (\*) indicate significant differences as determined by one-way ANOVA at a significance level of

$P < 0.05$ . **c** Principal component analysis (PCA) of bacterial community composition based on the Bray-Curtis distance metric. HY Hongyang, HWD Hayward, RS Rhizosphere soil, R Root endospheric samples, C Cane endospheric samples, L Leaf endospheric samples.



**Fig. 2 | The bacterial community composition in endophytic and rhizosphere soil samples of two kiwi cultivars. a** The relative abundance of the bacteria genera. Number showed the number of significantly ( $P < 0.05$ ) differentiated bacterial genera between 'Hongyang' and 'Hayward' by student's  $t$ -test. HY: Hongyang; HWD: Hayward. **b** Biomarker species in the rhizosphere soil of 'Hongyang' and

'Hayward' were identified through LEfSe analysis (LDA score  $>4$ ,  $P < 0.05$ ). Bacterial taxonomic at the genus level are denoted by the prefix g\_. c OTUs with a relative abundance greater than 0.5% in the *Flavobacterium* in rhizosphere soil. Error bars represent the standard deviation of three replicates, and significant differences (determined by ANOVA with a significance level of  $P < 0.05$ ) are denoted by \*.

compared to the non-treated control. The percentage inhibition of detached leaf discs was 98.2%, 45.7%, 62.1%, and 45.1%, respectively, while the percentage inhibition of detached canes was 88.2%, 76.0%, 79.6%, and 76.6%, respectively (Fig. 3d–g). Overall, treatment with these microbes provided protection against *Psa* infection compared to the control group without treatment, with F55 exhibiting the most potent inhibitory effect. The F55 isolates were subjected to further testing to assess their effectiveness in controlling *Psa*-induced disease in the canes and petioles of whole kiwifruit plants. The canes and petioles were treated with F55 prior to *Psa* inoculation. This intervention provided significant protection, resulting in an 87.7% reduction in infection in the canes and an 83.5% reduction in the petioles compared to the untreated control (Fig. 3h–k).

To confirm whether the above-described *Flavobacterium* isolates could prevent *Psa* infection in the field, we carried out the respective assay by selecting F55 as a representative. In the field trial, the biocontrol efficiency of KBC on flowers was evaluated during the flowering period in early May of the second year of application. The control effect of strain F55 against KBC was 84.4%, significantly higher than kasugamycin's 69.0%, which was a predominant chemical for KBC control. There was no significant difference with *Bacillus subtilis* Ba168 (Table 1).

To understand how F55 (GenBank Assembly ID: PRJNA1094473) exhibits the best biocontrol effects at the molecule levels, we conducted a whole-genome assembly of F55 by integrating second and third-generation sequencing data. The Nanopore sequencing yielded 147,727 high-quality reads after filtration, with an average read length of 11,713 bp and an N50 read length of 15,167 bp. Additionally, Illumina sequencing generated around 979 Mega base pair of clean data with a Q30 value of 89.1%. The complete genome of F55 consists of a circular chromosome sequence of 5,360,377 bp with a G + C content of 34.67%. A total of 4619 coding genes have been identified within its genome (Fig. 4a). The genome sequence of F55 exhibits a close relationship to *Flavobacterium soyae* (Supplementary Fig. 2). Moreover, functional annotation using the CAZY database predicted the presence of 332 protein sequences in F55, including lysozyme, glycoside hydrolases, and chitinase (Supplementary Data 1). Secondary metabolic gene cluster analysis by antiSMASH revealed seven clusters in the F55 genome, including arylpolyenes, betalactones, ranthipeptides, terpenes, resorcinols, NRPS/NRPS-like/T1PKS, and proteusins (Supplementary Table 4). Furthermore, the KEGG database annotation revealed 4087 protein sequences, genes encoding proteins involved in nitrogen metabolism,

biosynthesis of siderophore group nonribosomal peptides, 1-aminocyclopropane-1-carboxylate (ACC) deaminase for promote plant growth, and lipopolysaccharide (LPS) biosynthesis for plant immune. F55 has a quorum sensing system, biofilm formation, and chemotaxis, which might contribute to its adaptation and fitness in the rhizosphere soil (Fig. 4a and Supplementary Data 2). To assess the growth-promoting potential of F55, kiwifruit seedlings were inoculated with the bacterium in a controlled growth chamber. The application of F55 significantly enhanced kiwifruit seedling growth (Fig. 4b–d). In comparison to the control, kiwifruit seedlings exhibited a substantial increase in fresh weight and plant height by 63.7% and 110%, respectively, on the 30th day after inoculation with F55. These findings imply that F55 may have multiple functions in safeguarding kiwi plants from *Psa* infection.

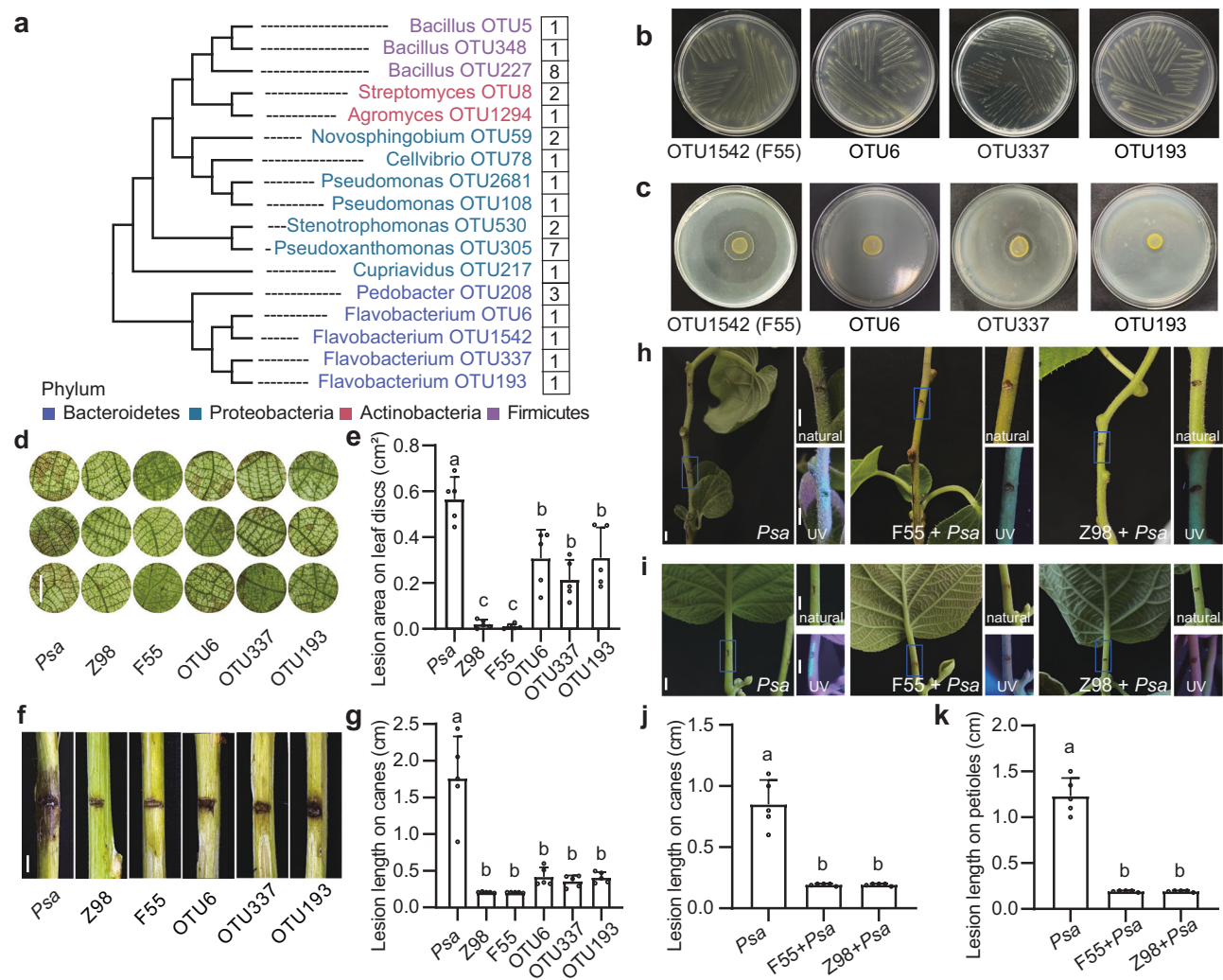
### The root exudates of resistant cultivar 'Hayward' promote *Flavobacterium soyae* F55 growth

To explore how the beneficial *Flavobacterium* isolates are enriched in the rhizosphere soil of 'Hayward', we carried out in vitro assays using F55, which demonstrated the highest biocontrol efficiency in detached leaves and canes. Seedlings of 'Hongyang' and 'Hayward' were cultured using tissue culture techniques, and their root exudates were collected to evaluate their influence on the growth and swimming ability of F55 in PDB medium. The results showed that the growth rate of F55 in Hayward's root exudates medium was significantly higher than that in Hongyang's root exudates medium and control at 36 h, 48 h, 60 h, 72 h, and 84 h (Fig. 5a). In addition, the application of 'Hayward' root exudates significantly enhanced the colonization-associated swimming motility of strain F55 compared with the application of 'Hongyang' root exudates and negative control (Fig. 5b). This suggests a potential mechanism by which 'Hayward' plants influence microbial behavior in the rhizosphere. Future studies could explore whether specific compounds in 'Hayward' root exudates induce the expression of motility-related genes in F55, thereby enhancing its colonization ability.

### Discussion

The rhizosphere is a vital component for plants, housing approximately  $10^{11}$  microbial cells per gram of rhizosphere soil<sup>5</sup>. These microbes play a critical role in plant health and offer several benefits such as enhancing nutrient uptake, modulating host immunity, and preventing pathogen colonization. Numerous studies have demonstrated that plants influence the composition





**Fig. 3 | Inhibition effect of *Flavobacterium* isolates against *Psa*.** **a** Construction of an evolutionary tree based on OTUs identified from 16S metabarcoding data corresponding to 35 isolates. The numbers in the square on the right indicate the number of isolated, exhibiting a similarity greater than 99% to the OTU sequences. **b** Morphological characteristics of *Flavobacterium* isolates in PDA medium. **c** Antibacterial activities of *Flavobacterium* OTU1542 (F55), OTU6, OTU337, and OTU193 against *Psa*. **d** Phenotypic observations of the control effects of different treatments on *Psa*-induced bacterial canker in 'Hongyang' kiwifruit leaf discs. **e** Evaluation of the impact of *Psa* inoculation on the lesion area of 'Hongyang' leaf discs following treated/untreated with above *Flavobacterium* OTUs. **f** Phenotypic observations of the control effects of different treatments on *Psa*-induced bacterial canker in 'Hongyang' kiwifruit detached canes. **g** Assessment of the impact of *Psa*

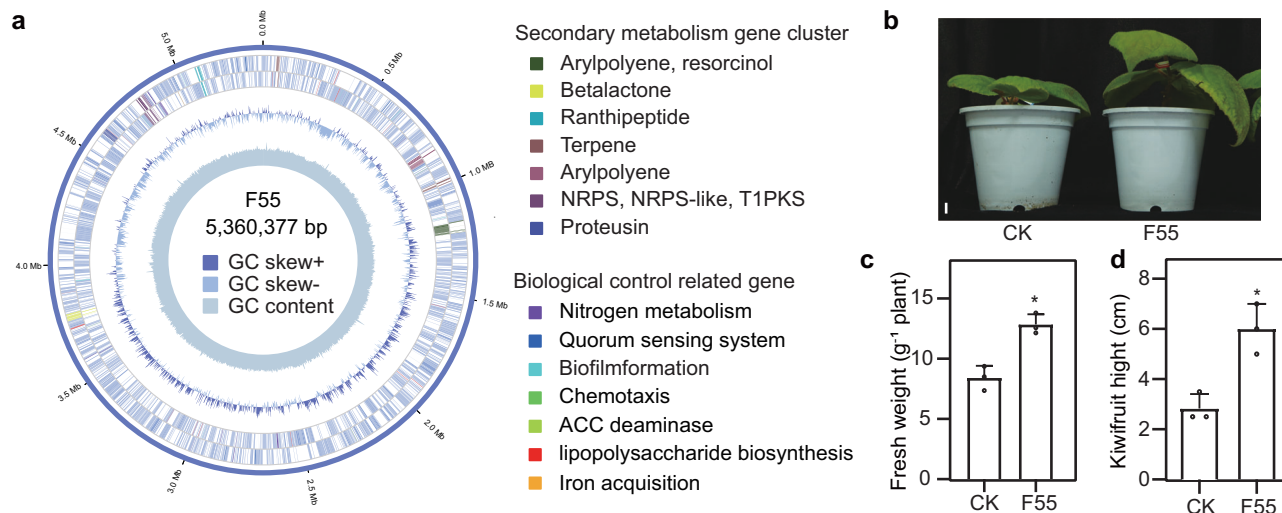
inoculation on the lesion length of 'Hongyang' detached canes following treated/untreated with above *Flavobacterium* OTUs. **h, i** Phenotypic observations of the control effects of different treatments on *Psa*-induced bacterial canker in potted 'Hongyang' kiwifruit canes and petioles. **j, k** Assessment of the impact of *Psa* inoculation on the lesion length of potted 'Hongyang' canes and petioles following treatment or no treatment with the specified F55. *Psa*: negative control, only with GFPuv-labeled M228. Z98: positive control, a *pseudomonas* strain with antagonistic activity against *Psa*. Scale bar in (d), (f), (h), and (i) indicates 0.5 cm. Each vertical bar represents the standard error of the means for five replicates. Statistically significant differences (determined by one-way ANOVA with a significance level of  $P < 0.05$ ) are denoted by lowercase letters.

**Table 1 | Biocontrol effect of F55 against KBC in the field**

Treatment	Average incidence of diseased flowers (%)	Control effect (%)
F55	1.61	84.37 ± 3.77 b
Kasugamycin	3.27	68.98 ± 5.94 a
<i>Bacillus subtilis</i> Ba168	2.35	77.44 ± 2.91 ab
Control treatment (CK)	10.56	~

F55 was cultured in PDB medium for 3 days, then centrifuged to remove the medium, and finally diluted with sterile water to a concentration of  $10^8$  CFU mL<sup>-1</sup>. *Bacillus subtilis* Ba168 and Kasugamycin were obtained from a commercial source and applied at the recommended dosage. Data collection was carried out across three distinct orchards. The control effect in the table represents the mean values ± SD, and the lowercase letters in the last column indicate the significance of a 5% difference between treatments.

of their rhizosphere soil microbiome, leading to distinct microbial communities among different plant types in the same soil. When plants come under attack by insects or pathogens, they have the ability to recruit beneficial microorganisms for support. Different plant compartments exhibit varying microbial community structures, and beneficial microorganisms are presumed to function within the plant tissues from where they have colonized. For instance, Liu et al.<sup>14</sup> observed that *Stenotrophomonas rhizophila* (SR80) sourced from the rhizosphere soil can suppress the soil-borne pathogen *Fusarium pseudograminearum*<sup>14</sup>. Similarly, Li et al.<sup>15</sup> recently uncovered that the citrus phyllosphere responds to infection by the fungal pathogen *Diaporthe citri* by recruiting microbiome members belonging to *Sphingomonas* species, which prevent pathogen infection by inhibiting fungal spore germination and hyphal growth<sup>15</sup>. In this study, we observed that the kiwifruit canker-resistant cultivar 'Hayward' exhibited an

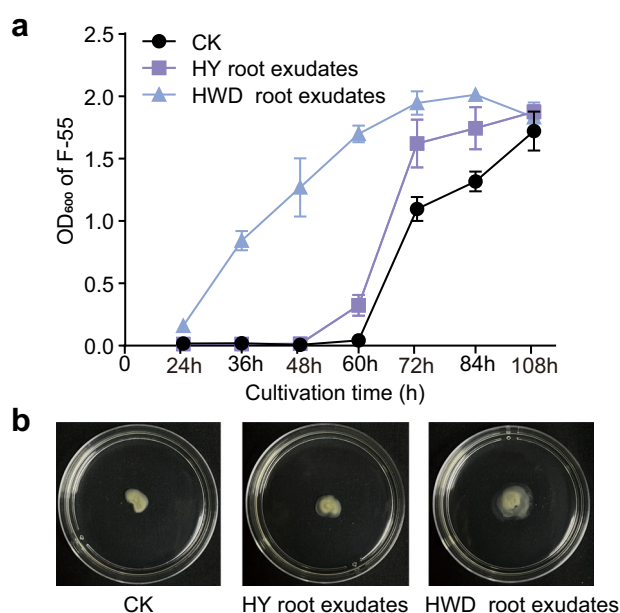


**Fig. 4 | Genome map and functional characteristics of *Flavobacterium soyae* F55.**

**a** Complete genome of F55. The circles (from outer to inner) represent genome sequence, CDSs on the forward and reverse, GC skew, and GC content. KEGG was used for annotating gene functions, genes encoding proteins involved in nitrogen metabolism (22 related genes), biosynthesis of siderophore group nonribosomal peptides (1 related gene), 1-aminocyclopropane-1-carboxylate (ACC) deaminase (1 related gene), lipopolysaccharide (LPS) biosynthesis (21 related genes), quorum sensing system (23 related genes), biofilm formation (18 related genes), and

chemotaxis (2 related genes). Anti-SMASH was used to predict secondary metabolic gene cluster, and 7 secondary metabolic related gene clusters were identified.

**b** Effects of F55 inoculation on kiwi plant growth. **c** Biomass accumulation of 'Hongyang' fresh weight 30 d after the treatment applications. CK: sterile Millipore water; F55: inoculated with F55 only. **d** Heights of kiwi plant 30 d after application of treatments. Scale bar indicates 1 cm. Statistically significant differences (determined by Student's *t*-test at a significance level of  $P < 0.05$ ) are denoted by asterisks (\*).



**Fig. 5 | Effects of addition root exudates on growth and swimming of F55.** **a** The root exudates can promote growth of F55 in PDB medium. HY Hongyang, HWD Hayward. **b** Plate assay showed root exudates can promote F55 motility in swimming medium. Each sample contains three biological replicates.

enrichment of *Flavobacterium* in the rhizosphere microbiome compared to the susceptible cultivar 'Hongyang'. We also demonstrated the positive effects of *Flavobacterium* in regulating above-ground kiwi canker diseases, thereby presenting a case study to highlight the cross-niche applications of rhizosphere soil bacteria from underground to above-ground (Fig. 6).

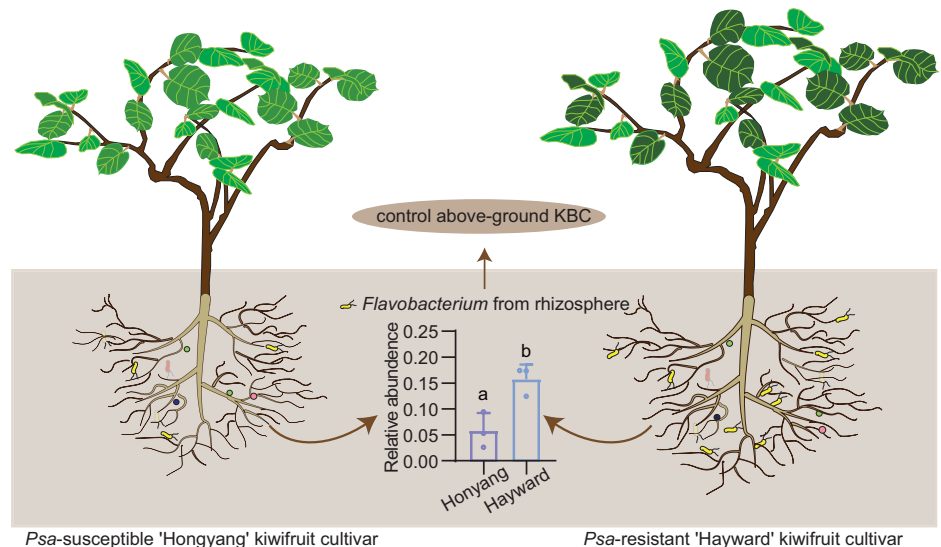
Recent studies have demonstrated that disease-resistant plants can recruit beneficial microbes, which aid them in withstanding external stresses such as adverse conditions and microbial invasion. Matsumoto et al.<sup>16</sup> observed an enrichment of *Sphingomonas* in the endophytes of

*Burkholderia plantarii*-resistant rice seeds, suggesting its potential role as a resistance-conferring agent<sup>16</sup>. In our investigation, we observed selective enrichment of distinct microbial communities in the rhizosphere soil of 'Hongyang' and 'Hayward'. Particularly noteworthy was the significant enrichment of the *Flavobacterium* in the rhizosphere soil of 'Hayward'. The rhizosphere serves as a critical interface for plant-microbe interactions, where plants release nutrients through root secretions, thereby facilitating diverse microbial functions. Previous reports have highlighted the ability of beneficial soil microorganisms to induce systemic resistance, enhancing plant defense mechanisms both above and below ground. For instance, Kwak et al.<sup>17</sup> demonstrated that a strain of the *Flavobacterium*, TRM1, significantly enriched in the rhizosphere soil of tomato plants resistant to *Ralstonia solanacearum*, suppressed bacterial wilt by activating plant immune-related genes<sup>17</sup>. However, our findings indicate that *Flavobacterium* F55, OTU6, OTU337, and OTU193 directly inhibit *Psa* in the above-ground parts of kiwi plants, thereby reducing the severity of KBC in antagonistic activity experiments. While the presence of an antagonistic *Flavobacterium* against *Psa* isolated from the kiwifruit rhizosphere has been previously documented, our study presents significant distinctions<sup>18</sup>. The earlier study employed traditional dual-culture methods, involving random screening of collected samples to identify strains with antagonistic activity. In contrast, our study utilizes a microbiome-guided, targeted approach to isolate bacterial biocontrol agents specific to woody plants. This method is exemplified by the isolation of the *Flavobacterium* strain F55 from the kiwifruit rhizosphere, which not only demonstrates anti-*Psa* activity but also provides crucial protection against *Psa* infection in kiwifruit plants—an aspect not covered in the previous research.

Some beneficial microorganisms have been reported to inhibit pathogens through the production of antibiotic compounds, nutrient competition, and lyase production. Accordingly, analysis of the genome of F55, revealed several genomic characteristics that could potentially benefit kiwi plants and inhibit pathogens. In particular, F55 might inhibit or kill *Psa* by producing enzymes such as lysozymes and glycoside hydrolases. Lysozymes have the ability to kill bacteria by hydrolyzing peptidoglycan in the cell wall, while glycoside hydrolases effectively disrupt bacterial membranes by degrading complex polysaccharides<sup>19,20</sup>. Moreover, the prediction of seven secondary metabolite synthesis gene clusters revealed the presence of

**Fig. 6 | A proposed mode illustrating how *Flavobacterium* enriched in the rhizosphere soil of *Psa*-resistant kiwi cultivar ‘Hayward’ defends against the above-ground canker caused by *Psa*.**

Compared to the *Psa*-susceptible kiwi cultivar ‘Hongyang’, the *Psa*-resistant kiwi cultivar ‘Hayward’ enriched more *Flavobacteria* in the rhizosphere soil, which could directly inhibit pathogenic *Psa* to maintain kiwi health.



terpenes, which have been reported to inhibit *Psa*<sup>21</sup>. The genome of F55 contains features such as quorum sensing and carbohydrate utilization, which aid in root tip colonization. In support, Carrión et al.<sup>22</sup> discovered that in soils suppressing *Rhizoctonia solani*, genes associated with signaling mechanisms were enriched in the rhizosphere soil of sugar beet. These specific gene enrichments were associated with endophytic microorganisms such as Chitinophaga, Flavobacteriaceae, and Pseudomonadaceae<sup>22</sup>. However, it is important to note that these characteristics are genomic and may not necessarily reflect functional expression. Further studies are required to investigate the functional expression of these genes and confirm their role in the inhibition mechanism.

Recent studies have demonstrated that plants can modulate microbial communities through both physical and chemical signals, including sugars, amino acids, and secondary metabolites<sup>23,24</sup>. For example, cucurbitacin B from melons can be released into the soil, selectively enriching genera such as *Enterobacter* and *Bacillus*, thereby suppressing *Fusarium oxysporum*<sup>25</sup>. In a recent investigation, researchers identified 4-hydroxycinnamic acid (4-HCA) as the primary driver for the proliferation of bacteria belonging to the Pseudomonadales order. This 4-HCA-mediated equilibrium has implications for host health<sup>26</sup>. The genetic determination of *Psa* resistance in the resistant cultivar and genetic modifications in kiwi plant can affect phytohormones and metabolism, thus influencing the composition of the microbial community<sup>27,28</sup>. The growth of F55 in the medium supplemented with ‘Hayward’ root exudate was significantly higher compared to that supplemented with ‘Hongyang’ root exudate. This indicates that the differences in the abundance of F55 in ‘Hongyang’ and ‘Hayward’ may be attributed to the root exudation from their respective host plants.

In summary, this study presents a case study highlighting cross-niche protection of kiwi plant from above-ground canker disease by beneficial rhizosphere soil microbiome bacteria. Utilizing the advantageous properties of rhizosphere *Flavobacterium* may present potential strategies to improve the growth and resilience of kiwi plants in agricultural settings.

## Methods

### Sample collection

The resistance of kiwifruit to *Psa* is influenced by genetic factors. Screening thousands of *Actinidia* genotypes from 24 taxa in PFR’s breeding program for resistance to *Psa* infections revealed that diploid (2×) *A. chinensis* var. *chinensis* is more susceptible than tetraploid (4×) *A. chinensis* var. *chinensis*, which, in turn, is more susceptible than hexaploid (6×) *A. chinensis* var. *deliciosa*<sup>29–31</sup>. Additionally, leaf stomatal density, stomatal length, and twig lenticel density show significant negative correlations with kiwi canker resistance<sup>32,33</sup>. In this study, five

commercial cultivars, *A. chinensis* var. *deliciosa* ‘Hayward’ (6×), *A. chinensis* var. *deliciosa* ‘Cuixiang’ (6×), *A. chinensis* var. *deliciosa* ‘Xuxiang’ (6×), *A. chinensis* var. *chinensis* ‘Hongyang’ (2×), and *A. chinensis* var. *chinensis* ‘Jinfu’ (6×), were selected for analysis of their resistance level to *Psa*. Among these, the lesion areas on leaf discs of the ‘Hongyang’ cultivar were significantly higher compared to the others, while ‘Hayward’ exhibited the smallest lesion areas (Supplementary Fig. 3). Therefore, ‘Hayward’ was considered the resistant cultivar, while ‘Hongyang’ was found to be susceptible to *Psa* in this study. Standard agricultural management practices were followed and no pesticides or fertilizers were applied for one month prior to sampling. Various factors such as marginal effects, appearance, pests and diseases, and vigorous plant growth were taken into consideration. All leaf, cane, root, and rhizosphere soil samples were collected from 7-year-old kiwi plants with no symptoms of KBC from the same orchard in Liuhuangbao village (34°17′24″N, 108°6′10″E), Yangling, Shaanxi province, China, in November 2019. The sampling occurred after fruit harvesting but prior to leaf shedding. To enhance reproducibility and normalization, ten uniform segments of kiwi cane, each approximately 1 cm in length, were selected to represent the entire cane. For the leaf samples, ten fully developed, mature leaves exhibiting a robust, deep green color were selected from each kiwi plant. Rhizosphere soil samples, each weighing over 3 grams, were collected from the coral-like fleshy roots at a depth of 10 to 20 cm below the soil surface, an area marked by significant root-soil microbial interactions. Following a thorough removal of excess soil through vigorous shaking, the fleshy roots were transferred into sterile 50 mL centrifuge tubes<sup>34</sup>. All tools, plastic bags, and gloves utilized during sample collection were sterilized to prevent contamination. Samples were promptly placed on ice and transported to the laboratory for analysis. The study focused on three replicate samples from both the ‘Hongyang’ and ‘Hayward’ cultivars for each compartment analyzed.

### Sample processing

The leaves and canes were sterilized by soaking them in sterile Millipore water for 30 s, followed by washing in 75% (v/v) ethanol for 2 min, and then in a sodium hypochlorite solution (2.5% active Cl<sup>−</sup> with 0.1% Tween 80) for 5 min. Finally, the sterilized samples were rinsed five times with sterile Millipore water. The roots collected above were placed into a 50 mL centrifuge tube containing 20 mL of sterile PBS buffer solution (130 mM NaCl, 7 mM Na<sub>2</sub>HPO<sub>4</sub>, 3 mM NaH<sub>2</sub>PO<sub>4</sub>, pH 7.4). They were then shaken at 120 rpm for 15 min to obtain the rhizosphere soil solution and the fleshy roots, respectively<sup>34</sup>. Next, the roots were sterilized following the same steps as those for the leaves and canes. The rhizosphere soil samples were obtained by centrifuging the rhizosphere soil solution in a tube at



12,000 rpm for 10 min. Finally, all samples were crushed and homogenized under aseptic conditions.

### 16S rRNA gene amplicon pyrosequencing and analysis

DNA extraction from rhizosphere soils, roots, canes, and leaves was performed according to the recommended protocol of QIAGEN GmbH, the manufacturer of the DNeasy® PowerSoil® Kit. DNA quality and concentrations were tested using 1% agarose gel electrophoresis and NanoDrop 1000 spectrophotometry (Thermo Scientific, Waltham, MA, USA). For community analysis, a portion of the 16S rRNA genes was amplified with barcoded universal primers 515 F and 907 R of the V3–V4 regions of the 16S rRNA gene<sup>35</sup>. A TruSeq® DNA PCR-Free Sample Preparation Kit (Illumina, USA) was used to generate the amplicon library, and sequencing was performed using a NovaSeq 6000 system (Illumina, Inc., CA, USA) by Novogene Biotechnology Co. Ltd.

Raw reads were assigned based on barcode sequences. After PCR amplification, primers sequences, and barcode sequences were removed, paired-end reads were assembled using FLASH (version 1.2.7)<sup>36</sup>. QIIME (Quantitative Insights Into Microbial Ecology, version 1.9.1) and its plugins were used for quality control to obtain effective tags, which included the elimination of low-quality reads and the removal of chimeras<sup>37</sup>. Furthermore, singleton sequences were discarded. The UPARSE-OTU algorithm in USEARCH was applied to cluster the acquired effective tags, ensuring a similarity of  $\geq 97\%$ , thus defining them as Operational Taxonomic Units (OTUs)<sup>38</sup>. The sequences with the highest frequency among OTUs were selected as representative sequences for species annotation analysis using Mothur based on the SILVA 132 SSU rRNA<sup>39,40</sup>. MUSCLE (version 3.8.31) was used for fast multi-sequence alignment to obtain the phylogenetic relationships of all OTUs<sup>41</sup>. Alpha diversity and beta diversity analysis were performed after standardizing the sample data, which were calculated through QIIME.

### Bacterial isolation and identification

A pure culture experiment was performed to recover the bacteria from the ‘Hayward’ rhizosphere soil using potato dextrose agar (PDA), Luria-Bertani (LB), and Trypticase Soy Broth (TSB) media. The ‘Hayward’ rhizosphere soil was homogenized with sterile PBS solution and heated at 28 °C for 15 min. Next, 50  $\mu$ L aliquots of a  $10^{-4}$  dilution of the rhizosphere soil suspension were coated onto sterile medium plates and cultured at 28 °C for 2–5 days. Single colonies were selected from the plates and further purified. Total bacterial DNA was extracted following the instructions provided by the FastPure® Blood/Cell/Tissue/Bacteria DNA Isolation Mini Kit (DC112; Vazyme Biotech Co. Ltd., Nanjing, China). PCR amplification of the 16S rRNA gene was performed using Es Taq Master Mix (Dye) (Cwbio Biotech Co. Ltd., Jiangsu, China) with the primer pair 27F and 1492R<sup>42</sup>. The PCR amplification program was as follows: 2 min at 94 °C; 30 cycles with a denaturation step of 30 s at 94 °C, 30 s at 58 °C, and 30 s at 72 °C; followed by a final step of 2 min at 72 °C. Purification and sequencing of PCR products were performed by Sangong Biotech Co. Ltd., Shanghai, China. The 16S rRNA gene sequences of the isolated strains were compared with the corresponding 16S rRNA amplicon sequences obtained from rhizosphere soil. Strains showing more than 99% sequence similarity were stored at -80 °C for subsequent experiments.

### Antagonistic activity test of the bacteria against *Psa*

The hypervirulent *Psa* strain M228 (WGS accession: ANJI02) was isolated from infected canes of *A. chinensis* var. *chinensis* ‘Hongyang’ in Shaanxi Province of China in 2010<sup>43</sup>. This strain was used as the target pathogen. The antagonistic activity of bacteria isolated from ‘Hayward’ rhizosphere soil against *Psa* was evaluated using the agar disc diffusion method<sup>44</sup>. The PDA solid medium was melted and cooled to 45 °C. Mixed plates were prepared by combining 1 mL of *Psa* M228 bacterial suspension ( $10^9$  CFU mL<sup>-1</sup>) with 100 mL of molten PDA. The mixture was then poured into Petri dishes to cool and solidify. Each test isolate (5  $\mu$ L,  $10^8$  CFU mL<sup>-1</sup>) from the ‘Hayward’ rhizosphere soil was

separately spotted in the center of the mixed media plates. The inhibition of *Psa* growth was assessed by measuring the diameter of the clear inhibition zones around the spots. The antibacterial zones were measured after incubation at 28 °C for 4 days. The experiment was conducted three times for reliability.

The in vitro tissue antagonistic activity of ‘Hayward’ rhizosphere isolates *Flavobacterium* OTU1542 (F55), *Flavobacterium* OTU6, *Flavobacterium* OTU337, and *Flavobacterium* OTU193 was evaluated using leaf-disk inoculation via vacuum infiltration and detached cane inoculation via cutting wounds<sup>43</sup>. ‘Hongyang’ leaves were disinfected with a sodium hypochlorite solution (0.6% active Cl<sup>-</sup>) for 6 min, followed by thorough rinsing with sterile Millipore water until no unpleasant odor was detected. After sterilization, leaf discs (1 cm in diameter) were prepared, and a ‘Hayward’ rhizosphere culture suspension ( $10^4$  CFU mL<sup>-1</sup>) were vacuum-infiltrated into each leaf disc. Subsequently, *Psa* M228 ( $10^4$  CFU mL<sup>-1</sup>) was inoculated 24 h later on the same discs using vacuum infiltration. Detached ‘Hongyang’ canes (with an approximate diameter of 0.8 cm, cut into segments ~12 cm in length) were surface sterilized by rising in a sodium hypochlorite solution (0.6% active Cl<sup>-</sup>) for 10 min, and soaked in sterile Millipore water three times (1 min each time). The sterilized cane ends were sealed with paraffin wax. A 0.2 cm wound was made in the middle of the canes using a sterile blade. The wound was inoculated with 10  $\mu$ L of the rhizosphere isolates ( $10^8$  CFU mL<sup>-1</sup>), followed 24 h later by 10  $\mu$ L of *Psa* M228 ( $10^8$  CFU mL<sup>-1</sup>). The canes were placed in a sterile seed tray and covered with cling film to maintain moisture. Both canes and leaf discs were placed in a plant growth chamber (at 95% relative humidity) under light for 16 h at 18 °C and darkness for 8 h at 14 °C<sup>44</sup>. Only *Psa* M228 inoculation was used as a negative control, while the positive control was a *pseudomonas* strain with strong antagonistic activity against *Psa*<sup>45</sup>. Lesion size on the leaves was measured after 4 days and the length of the lesion on the canes was measured after 20 days of culture in the artificial climate chamber. Five independent experiments were conducted.

The rhizosphere isolate F55, which showed the strongest in vitro tissue antagonistic activity, was further evaluated using whole potted kiwifruit plants. The canes and petioles of ‘Hongyang’ were surface sterilized with 75% ethanol and rinsed with sterile water until the alcohol odor was no longer detectable. Subsequent processing followed the detached cane inoculation via cutting wounds method, where a 0.1 cm wound was made in the middle of the canes and petioles using a sterile blade. The *Psa* inoculation was performed 24 h after the initial F55 inoculation. Both the F55 and *Psa* inoculations used a volume of 2.5  $\mu$ L. The length of the lesions on the canes and petioles was measured after 7 days. Five independent experiments were conducted.

### Biocontrol effects of strain F55

Field experiments were conducted in three distinct Chinese villages: Tianjiabao (34°12′52″N, 108°15′33″E) in Mei County, Shaanxi Province; Liuhuangbao (34°17′5″N, 108°5′44″E), and Shangde (34°15′25″N, 108°2′42″E) in Yangling, Shaanxi Province. The F55 suspension was prepared by dissolving F55 in sterile water to achieve a concentration of  $1 \times 10^8$  CFU mL<sup>-1</sup>. Subsequently, this 5L F55 suspension was sprayed onto each kiwi tree in the field. Specifically, the spraying schedule included applications before flowering, after flowering, after fruit harvesting, and before leaf shedding in the first year, and pre-flowering in the second year. During the second year’s flowering period, the rate of diseased flowers was calculated by determining the ratio of infected flowers to the total surveyed flowers. Disease assessment includes the identification of signs of decay or necrosis in flowers of *A. chinensis* cv. ‘Hongyang’ is used for field trials. Sterile water was used as a negative control, while the positive control contained *Bacillus subtilis* Ba168 (Yangling Ludu Biotechnology Co., Ltd., Shaanxi, China) and 6% kasugamycin (Shaanxi Sunger Road Bioscience Co., Ltd.) dissolved in sterile water, with concentrations prepared according to the producer’s instructions.

## Genome assembly and functional prediction of the strongest antagonistic activity strain F55

Whole genome sequencing, assembly, and functional analysis of strain F55 were performed. The strain F55 was cultured in PDB medium for 72 h, centrifuged at 12,000 rpm for 2 min, and washed three times with PBS buffer after centrifugation to remove the medium. Genomic DNA extraction, assessment, library construction, and subsequent sequencing were performed by Novegene Biotechnology Co., Ltd. in Beijing, China. Illumina NovaSeq PE150 (Illumina, San Diego, CA, USA) and Nanopore PromethION (Oxford, UK) library technologies were used. The PromethION file was converted to fastq file and underwent base-calling through GUPPY (version 5.0.16). Assembly of the second-generation Illumina and third-generation Nanopore sequencing data was performed using Unicycler (version 0.4.8) after quality control. For prediction, functional information of strain F55, gene functional annotation was performed using Kyoto Encyclopedia of Genes and Genomes (KEGG) and the Carbohydrate-Active enZymes Database (CAZy)<sup>46,47</sup>. Prediction of secondary metabolism biosynthesis gene clusters was performed using anti-SMASH (version 4.0.2)<sup>48</sup>.

## Plant growth-promoting test of strain F55

To avoid interference from endophytic microorganisms and rootstocks, tissue culture seedlings were used in pot experiments to evaluate the plant growth-promoting effect of strain F55. ‘Hayward’ and ‘Hongyang’ leaves were inoculated onto a callus induction medium (MS-modified medium containing vitamins, sucrose, and agar) supplemented with 1 mg L<sup>-1</sup> thiazuron and 0.15 mg L<sup>-1</sup> indole-3-butyric acid for 20 days<sup>49</sup>. Adventitious buds were induced in MS modified medium supplemented with 3 mg L<sup>-1</sup> 6-benzylaminopurine and 0.1 mg L<sup>-1</sup> 1-naphthylacetic acid. The regenerated kiwi plants were then transferred to 1/2 MS-modified medium supplemented with 0.6 mg L<sup>-1</sup> indole-3-butyric acid and 0.3 mg L<sup>-1</sup> indole-3-acetic acid to promote root development. Both MS and 1/2 MS modified media were purchased from Coolaber Co. Ltd (Beijing, China). Kiwi shoots were cultured in 250 mL plastic vessels containing 50 mL of medium each. The kiwi plants were cultivated in a plant growth chamber at a temperature of 26 ± 1 °C (16 h light and 8 h dark). Seedlings with well-developed roots were gently washed in sterilized water, transferred to sterilized pots containing commercial substrate, and covered with a plastic lid to retain moisture for one week before removing the cover.

Microbial biocontrol agents are known to protect plants by directly eliminating pathogens and/or promoting plant growth<sup>50</sup>. Given that we observed the direct inhibitory effect of F55 on *Psa* growth, we subsequently investigated its potential to enhance kiwi plant growth. The growth-promoting effect of strain F55 on kiwi seedlings was evaluated in a plant growth chamber. Aseptic ‘Hongyang’ seedlings with uniform growth were obtained and cultured successfully. Bacterial suspensions containing F55 were added to pots filled with sterile pindstrup substrate (Pindstrup Mosebrug A/S, Ryomgård, Denmark) at a density of 10<sup>8</sup> CFU g<sup>-1</sup>. Sterile Millipore water was used as the control. Each experiment was replicated three times. Seedling growth parameters, such as plant height and fresh weight, were evaluated after one month and compared between the bacterial-inoculated and non-inoculated samples.

## Growth promotion and motility effects of root exudate on strain F55

Tissue culture seedlings of the ‘Hongyang’ and ‘Hayward’, aged three months, were collected with their roots and cultured as described in the plant growth-promoting test. To prevent root damage, the roots were gently rinsed with running water to remove any adhering substrate. The kiwi seedlings were immersed in a 0.6% active Cl<sup>-</sup> sodium hypochlorite solution for 5 min to disinfect the surface, followed by three rinses with

sterile water for 30 s each. Subsequently, the entire seedlings were placed in a conical flask with 500 mL of sterile Millipore water, with only the root system immersed, and cultured for 3 days under the aforementioned conditions. Afterward, the kiwi seedlings were removed to obtain the root exudate solution. The collected root exudate solution was filtered through a 0.22 µm filter (Millipore)<sup>51</sup>. The root exudate solution was concentrated from 1 L to 20 mL through freeze-drying, and subsequently stored at -80 °C.

Each 3 mL of root exudate solution was diluted with 300 mL of PDB medium to prepare the root exudate medium. Next, 200 µL of F55 suspension (10<sup>8</sup> CFU mL<sup>-1</sup>) was added to the root exudate medium, shaking at 220 rpm, 28 °C. PDB medium without the addition of root exudate solution was used as a negative control. The optical density (OD) values of the bacterial cultures were measured at different time intervals (24 h, 36 h, 48 h, 60 h, 72 h, 84 h, 108 h) using a UV-visible spectrophotometer at a wavelength of 600 nm<sup>52</sup>. This analysis aimed to evaluate the influence of root exudates on strain F55 growth. Each experimental condition was replicated three times.

Bacterial solutions 2.5 µL (3 × 10<sup>8</sup> CFU mL<sup>-1</sup>) were stabbed into the center of the swimming medium (PDB medium with 0.25% agar supplemented with 1% ‘Hongyang’/‘Hayward’ root exudate solution). No addition of root exudate was used as a negative control. Inoculation was performed 72 h later to assess bacterial motility in the swimming medium. Each experiment was performed three times.

## Statistics and reproducibility

The analysis was performed using GraphPad Prism version 8.0.0 (San Diego, California, USA). Normal distribution of data was assessed using the Shapiro-Wilk test, and homogeneity of variances was confirmed with either the F-test or Brown-Forsythe test. Mean differences between treatments were analyzed using either Student’s *t*-test or Tukey’s multiple comparisons test. Post hoc comparisons were conducted using pairwise Wilcoxon rank-sum tests or Dunn’s multiple comparisons test, as appropriate. The linear discriminant analysis effect size (LEfSe) statistical analysis (<http://huttenhower.sph.harvard.edu/lefse/>) was used for biomarker analysis, with LDA scores greater than 4 considered significant (LDA > 4)<sup>53</sup>. Hierarchical clustering (based on Bray-Curtis dissimilarity matrix) and Principal Component Analysis (PCA) were performed to explore the structure and variability of microbial communities. Permutational Multivariate Analysis of Variance (PERMANOVA) was conducted to assess whether sample groups harbored significantly different microbial communities. These analyses were conducted using R (The R Foundation for Statistical Computing, Vienna, Austria)<sup>54</sup>. Sample sizes and the number of replicates are reported in the figure legends. Regarding reproducibility, we conducted five biological replicates for the antagonistic activity of ‘Hayward’ rhizosphere isolates in both in vitro tissue and whole potted kiwifruit plants. For the 16S rRNA gene amplicon pyrosequencing, plant growth-promoting tests, and the effects of root exudates on growth promotion and motility, we used three biological replicates.

## Reporting summary

Further information on research design is available in the Nature Portfolio Reporting Summary linked to this article.

## Data availability

The genome sequence of F55 has been deposited in the NCBI BioProject databases with accession code PRJNA1094473. Raw data of amplicon sequencing have been deposited in the NCBI BioProject databases with accession code PRJNA1097213. Other source data can be found in the file Supplementary Data 3.

Received: 3 April 2024; Accepted: 4 November 2024;  
Published online: 07 November 2024



## References

1. Zheng, D., Liwinski, T. & Elinav, E. Interaction between microbiota and immunity in health and disease. *Cell. Res.* **30**, 492–506 (2020).
2. Turner, T. R., James, E. K. & Poole, P. S. The plant microbiome. *Genome Biol.* **14**, 209 (2013).
3. 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).
4. Banerjee, S. & van der Heijden, M. G. A. Soil microbiomes and one health. *Nat. Rev. Microbiol.* **21**, 6–2 (2023).
5. Berendsen, R. L., Pieterse, C. M. & Bakker, P. A. The rhizosphere microbiome and plant health. *Trends Plant. Sci.* **17**, 478–48 (2012).
6. Bulgarelli, D., Schlaeppi, K., Spaepen, S., Ver Loren van Themaat, E. & Schulze-Lefert, P. Structure and functions of the bacterial microbiota of plants. *Annu. Rev. Plant. Biol.* **64**, 807–838 (2013).
7. Gong, C. et al. A QTL of eggplant shapes the rhizosphere bacterial community, co-responsible for resistance to bacterial wilt. *Hortic. Res.* **11**, uhad272 (2024).
8. Yin, J. et al. Heritability of tomato rhizobacteria resistant to *Ralstonia solanacearum*. *Microbiome* **10**, 227 (2022).
9. Ward, C. & Courtney, D. Kiwifruit: taking its place in the global fruit bowl. *Adv. Food Nutr. Res.* **68**, 1–14 (2013).
10. Scortichini, M., Marcelletti, S., Ferrante, P., Petriccione, M. & Firrao, G. *Pseudomonas syringae* pv. *actinidiae*: a re-emerging, multi-faceted, pandemic pathogen. *Mol. Plant. Pathol.* **13**, 631–640 (2012).
11. Vanneste, J., Moffat, B. J. & Oldham, J. M. Survival of *Pseudomonas syringae* pv. *actinidiae* on *Cryptomeria japonica*, a non-host plant used as shelter belts in kiwifruit orchards. *N. Z. Plant Prot.* **65**, 1–7 (2012).
12. Luo, J. et al. Advancements in the use of bacteriophages to combat the kiwifruit canker phytopathogen *Pseudomonas syringae* pv. *Actinidia*. *Viruses* **14**, 12 (2022).
13. Kim, G. H., Jung, J. S. & Koh, Y. J. Occurrence and epidemics of bacterial canker of kiwifruit in Korea. *Plant. Pathol. J.* **33**, 351–361 (2017).
14. Liu, H. et al. Evidence for the plant recruitment of beneficial microbes to suppress soil-borne pathogens. *New. Phytol.* **229**, 2873–2885 (2021).
15. Li, P. D. et al. The phyllosphere microbiome shifts toward combating melanose pathogen. *Microbiome* **10**, 56 (2022).
16. Matsumoto, H. et al. Bacterial seed endophyte shapes disease resistance in rice. *Nat. Plants* **7**, 60–72 (2021).
17. Kwak, M. J. et al. Rhizosphere microbiome structure alters to enable wilt resistance in tomato. *Nat. Biotechnol.* **36**, 1100–1109 (2018).
18. Yan, Z., Fu, M., Mir, S. H. & Zhang, L. Diversity and characterization of antagonistic bacteria against *Pseudomonas syringae* pv. *actinidiae* isolated from kiwifruit rhizosphere. *FEMS Microbiol. Lett.* **370**, fnad078 (2023).
19. Fleming, D., Chahin, L. & Rumbaugh, K. Glycoside hydrolases degrade polymicrobial bacterial biofilms in wounds. *Antimicrob. Agents Chemother.* **61**, e01998–01916 (2017).
20. Ragland, S. A. & Criss, A. K. From bacterial killing to immune modulation: Recent insights into the functions of lysozyme. *PLoS Pathog.* **13**, e1006512 (2017).
21. Guan, S. et al. Pitsubcosides A–L, highly esterified eudesmane sesquiterpenoid glycosides with antibacterial activity from *Pittosporum subulisepalum* and their mechanism. *Pest. Manag. Sci.* **79**, 3471–3485 (2023).
22. Carrión, V. J. et al. Pathogen-induced activation of disease-suppressive functions in the endophytic root microbiome. *Science* **366**, 606–612 (2019).
23. Foster, K. R., Schluter, J., Coyte, K. Z. & Rakoff-Nahoum, S. The evolution of the host microbiome as an ecosystem on a leash. *Nature* **548**, 43–51 (2017).
24. Raaijmakers, J. M. & Kiers, E. T. Rewilding plant microbiomes. *Science* **378**, 599–600 (2022).
25. Zhong, Y. et al. Root-secreted bitter triterpene modulates the rhizosphere microbiota to improve plant fitness. *Nat. Plants* **8**, 887–896 (2022).
26. Su, P. et al. Microbiome homeostasis on rice leaves is regulated by a precursor molecule of lignin biosynthesis. *Nat. Commun.* **15**, 23 (2024).
27. Olanrewaju, O. S., Ayangbenro, A. S., Glick, B. R. & Babalola, O. O. Plant health: feedback effect of root exudates-rhizobiome interactions. *Appl. Microbiol. Biotechnol.* **103**, 1155–1166 (2019).
28. Nunes da Silva, M. et al. Defence-related pathways, phytohormones and primary metabolism are key players in kiwifruit plant tolerance to *Pseudomonas syringae* pv. *actinidiae*. *Plant Cell. Environ.* **45**, 528–541 (2022).
29. Hoyte, S. et al. Developing and using bioassays to screen for *Psa* resistance in New Zealand kiwifruit. *Acta Hortic.* **1095**, 171–180 (2013).
30. De Silva, N. H., Gea, L. & Lowe, R. Genetic analysis of resistance to *Pseudomonas syringae* pv. *actinidiae* (*Psa*) in a kiwifruit progeny test: an application of generalised linear mixed models. *SpringerPlus* **3**, 547 (2014).
31. Tahir, J. et al. Multiple quantitative trait loci contribute to resistance to bacterial canker incited by *Pseudomonas syringae* pv. *actinidiae* in kiwifruit (*Actinidia chinensis*). *Hortic. Res.* **6**, 101 (2019).
32. Tu, M. et al. Study on the differences in resistance to canker disease and physiological mechanisms among six kiwifruit varieties. In: *Proceedings of the 7th National Kiwifruit Symposium*, 233–241 (2018).
33. Spinelli, F., Donati, I., Vanneste, J. L., Costa, M. & Costa, G. Real time monitoring of the interactions between *pseudomonas syringae* pv. *actinidiae* and *actinidia* species. *Acta Hortic.* **913**, 461–465 (2011).
34. Beckers, B., Op De Beeck, M., Weyens, N., Boerjan, W. & Vangronsveld, J. Structural variability and niche differentiation in the rhizosphere and endosphere bacterial microbiome of field-grown poplar trees. *Microbiome* **5**, 25 (2017).
35. Ludwig, W. Nucleic acid techniques in bacterial systematics and identification. *Int. J. Food Microbiol.* **120**, 225–236 (2007).
36. Magoc, T. & Salzberg, S. L. FLASH: fast length adjustment of short reads to improve genome assemblies. *Bioinformatics* **27**, 2957–2963 (2011).
37. Caporaso, J. G. et al. QIIME allows analysis of high-throughput community sequencing data. *Nat. Methods* **7**, 335–336 (2010).
38. Edgar, R. C. UPARSE: highly accurate OTU sequences from microbial amplicon reads. *Nat. Methods* **10**, 996–998 (2013).
39. Pruesse, E. et al. SILVA: a comprehensive online resource for quality-checked and aligned ribosomal RNA sequence data compatible with ARB. *Nucleic Acids Res.* **35**, 7188–7196 (2007).
40. Schloss, P. D. et al. Introducing mothur: open-source, platform-independent, community-supported software for describing and comparing microbial communities. *Appl. Environ. Microbiol.* **75**, 7537–7541 (2009).
41. Edgar, R. C. MUSCLE: multiple sequence alignment with high accuracy and high throughput. *Nucleic Acids Res.* **32**, 1792–1797 (2004).
42. Lane, D. J. 16S/23S rRNA sequencing. In E. Stackebrandt, M. Goodfellow (ed.) *Nucleic Acid Techniques in Bacterial Systematics*. 115–175 (John Wiley & Sons, 1991).
43. Zhao, Z. et al. Comparative genomics reveal pathogenicity-related loci in *Pseudomonas syringae* pv. *actinidiae* biovar 3. *Mol. Plant Pathol.* **20**, 923–942 (2019).
44. Wang, H. et al. *Paenibacillus polymyxa* YLC1: a promising antagonistic strain for biocontrol of *Pseudomonas syringae* pv. *actinidiae*, causing kiwifruit bacterial canker. *Pest. Manag. Sci.* **79**, 4357–4366 (2023).
45. Tian, Y. Antagonistic Effects and Mechanisms of *Flavobacterium* F-55 and *Pseudomonas* Z-98 against *Pseudomonas syringae* pv. *actinidiae* [Master's thesis] (Northwest A&F University, 2024).

46. Kanehisa, M., Goto, S., Kawashima, S., Okuno, Y. & Hattori, M. The KEGG resource for deciphering the genome. *Nucleic Acids Res.* **32**, D277–D280 (2004).
47. Cantarel, B. L. et al. The carbohydrate-active enZymes database (CAZy): an expert resource for glycogenomics. *Nucleic Acids Res.* **37**, D233–D238 (2009).
48. Medema, M. H. et al. antiSMASH: rapid identification, annotation and analysis of secondary metabolite biosynthesis gene clusters in bacterial and fungal genome sequences. *Nucleic Acids Res.* **39**, W339–W346 (2011).
49. Wu, J. et al. Transcriptome analysis of the salt-treated *Actinidia deliciosa* (A. Chev.) C. F. Liang and A. R. Ferguson plantlets. *Curr. Issues Mol. Biol.* **45**, 3772–3786 (2023).
50. Syed Ab Rahman, S. F., Singh, E., Pieterse, C. M. J. & Schenk, P. M. Emerging microbial biocontrol strategies for plant pathogens. *Plant Sci.* **267**, 102–111 (2018).
51. Vives-Peris, V., de Ollas, C., Gómez-Cadenas, A. & Pérez-Clemente, R. M. Root exudates: from plant to rhizosphere and beyond. *Plant Cell Rep.* **39**, 3–17 (2019).
52. Wen, T., Zhao, M., Yuan, J., Kowalchuk, G. A. & Shen, Q. Root exudates mediate plant defense against foliar pathogens by recruiting beneficial microbes. *Soil. Ecol. Lett.* **3**, 42–51 (2020).
53. Segata, N. et al. Metagenomic biomarker discovery and explanation. *Genome Biol.* **12**, R60 (2011).
54. Oksanen, J. et al. Vegan: Community Ecology Package. R package v.2.5-6 (2019).

## Acknowledgements

This work was supported by the National Key R&D Program of China (2022YFD1400200), the Special Support Plan for High-level Talent of Shaanxi Province and National Natural Science Foundation of China (32100148).

## Author contributions

L.H. designed the research. W.Z. and N.W. performed the experiments. W.Z., L.H., and N.W. analyzed the data. W.Z., N.W., G.Q., X.Q., W.L., and L.H. wrote the manuscript. All authors discussed the results and commented on the manuscript before submission.

## Competing interests

The authors declare no competing interest.

## Additional information

**Supplementary information** The online version contains supplementary material available at <https://doi.org/10.1038/s42003-024-07208-z>.

**Correspondence** and requests for materials should be addressed to Lili Huang.

**Peer review information** *Communications Biology* thanks Chidam Mahadevan, Nhu Nguyen, and the other, anonymous, reviewer for their contribution to the peer review of this work. Primary Handling Editor: David Favero. A peer review file is available.

**Reprints and permissions information** is available at <http://www.nature.com/reprints>

**Publisher's note** Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

**Open Access** This article is licensed under a Creative Commons Attribution-NonCommercial-NoDerivatives 4.0 International License, which permits any non-commercial use, sharing, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons licence, and indicate if you modified the licensed material. You do not have permission under this licence to share adapted material derived from this article or parts of it. The images or other third party material in this article are included in the article's Creative Commons licence, unless indicated otherwise in a credit line to the material. If material is not included in the article's Creative Commons licence and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this licence, visit <http://creativecommons.org/licenses/by-nc-nd/4.0/>.

© The Author(s) 2024