

Supplementary Materials

The Co-Association of Enterobacteriaceae and *Pseudomonas* with Specific Resistant Cucumber against *Fusarium* Wilt Disease

Yu-Lu Zhang, Xiao-Jing Guo, Xin Huang, Rong-Jun Guo, Xiao-Hong Lu and Shi-Dong Li, Hao Zhang

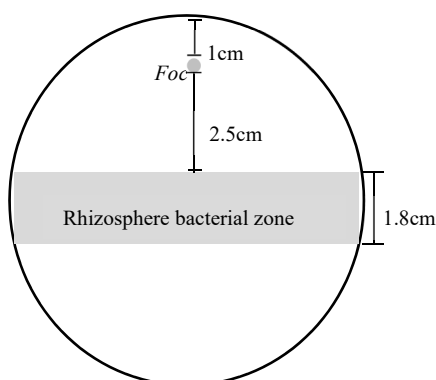


Figure S1. Demonstration of the isolation strategy of antagonistic bacteria.

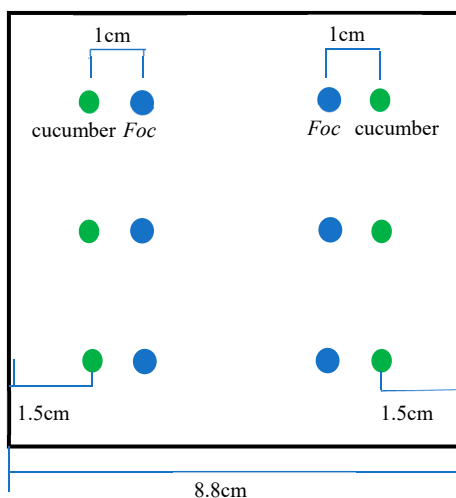


Figure S2. Demonstration of *Foc* inoculation site to the cucumber seedlings grown in bacteria inoculated soil.

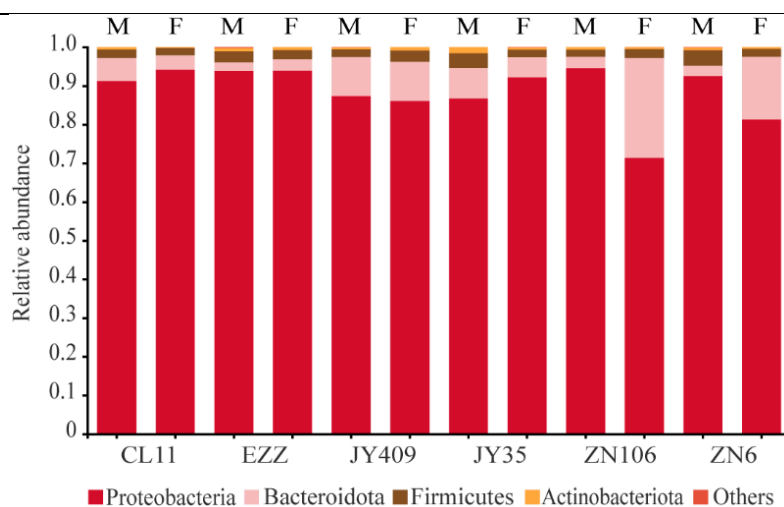


Figure S3. Taxonomic profiling of the bacterial communities in cucumber root across cultivars and treatments at phylum level. Phyla with a proportion of less than 1.0% are combined into the group “Others”. CL11, EZZ and JY409 are resistant cultivars, JY35 and ZN106 are moderately resistant cultivars, and ZN6 is a susceptible cultivar. M and F indicate mock and *Foc* inoculation, respectively.

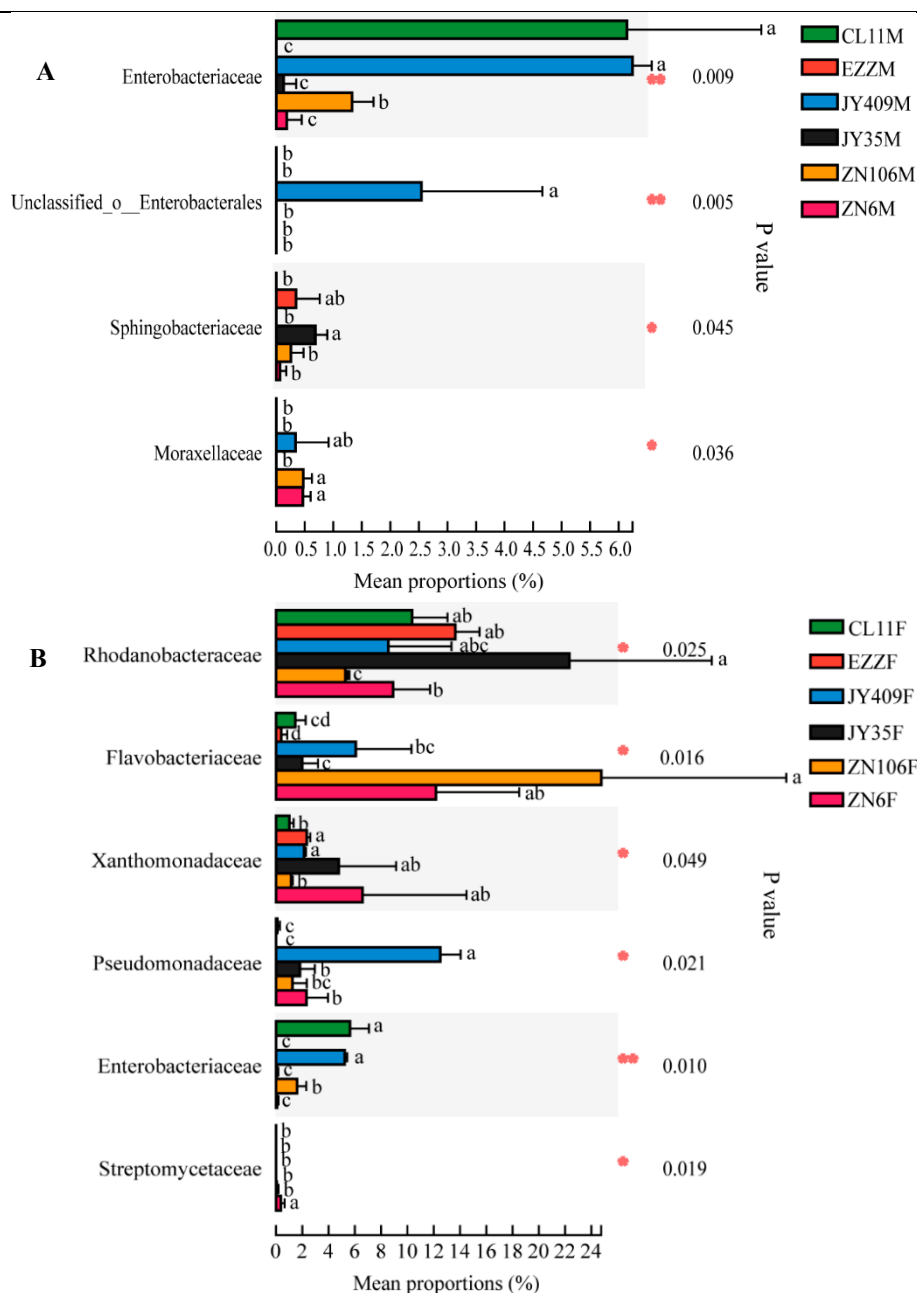


Figure S4. Differential bacterial phyla among cucumber cultivars with mock (A) and *Foc* inoculation (B). Data were tested by Kruskal-Wallis H test ($p < 0.05$) and validated by False Discovery Rate (FDR) and Tukey-Kramer with a confidence level of 0.95. *, $0.01 < p \leq 0.05$; **, $0.001 < p \leq 0.01$. Different letters beside the error bars of each phylum indicate significant difference among cultivars ($p < 0.05$).

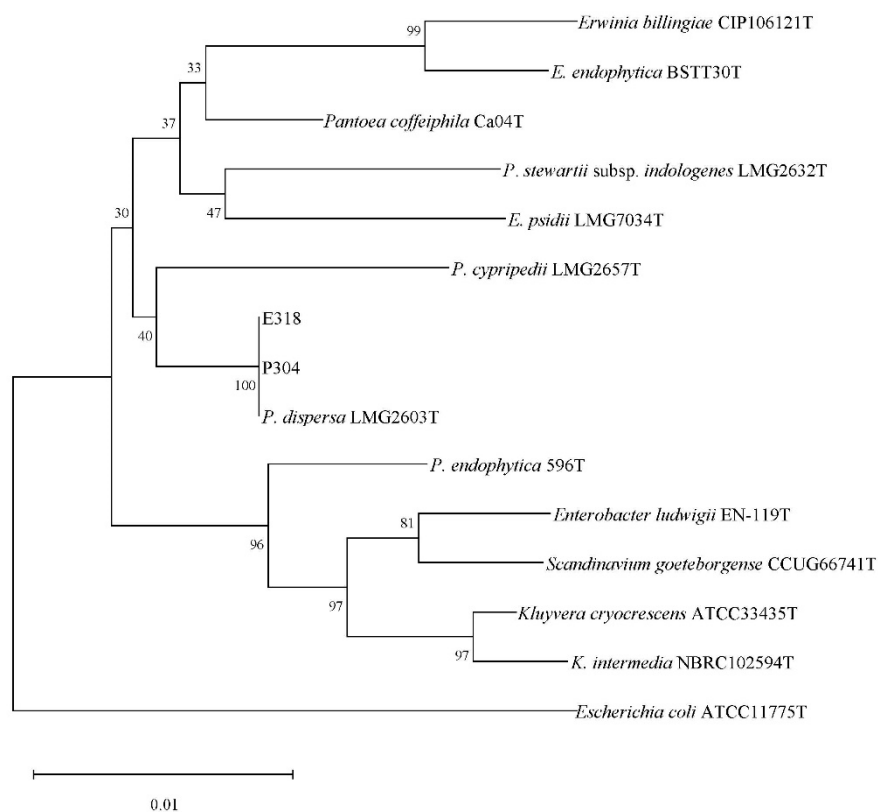


Figure S5. Phylogenetic trees of strain P304 and E318 based on their 16S rRNA gene sequences determined by the neighbor-joining method with the program package MEGA 11.0. Bootstrap confidence values were obtained using 2000 resamplings. Bar, 0.01 substitutions per site.

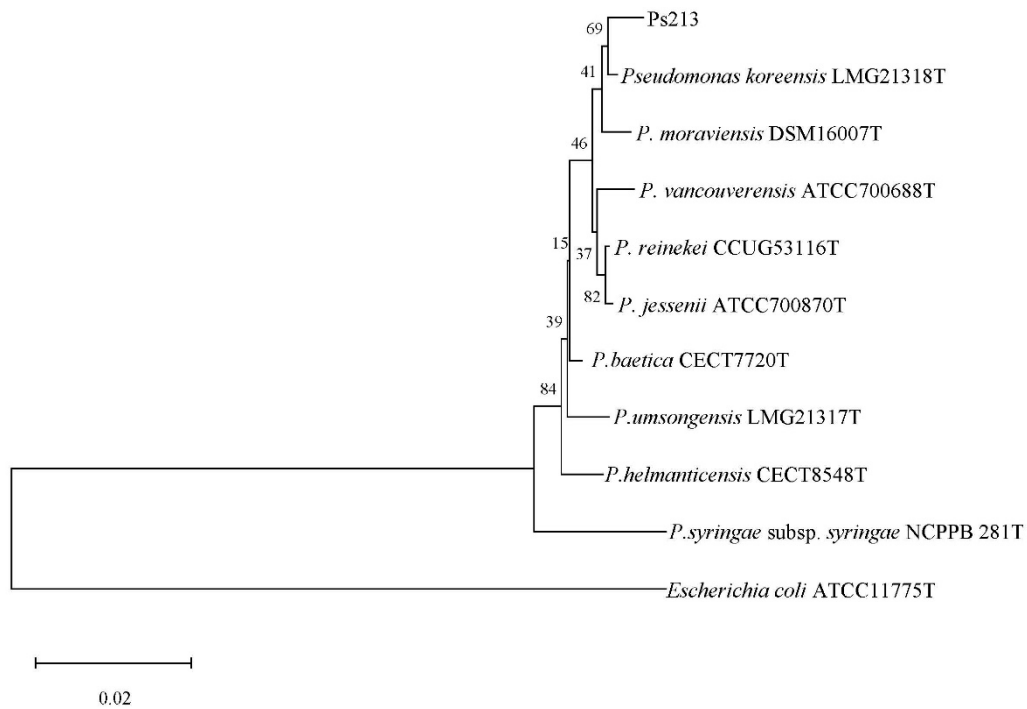


Figure S6. Phylogenetic tree of strain Ps213 based on its 16S rRNA gene sequence determined by the neighbor-joining method with the program package MEGA 11.0. Bootstrap confidence values were obtained using 2000 resamplings. Bar, 0.02 substitutions per site.

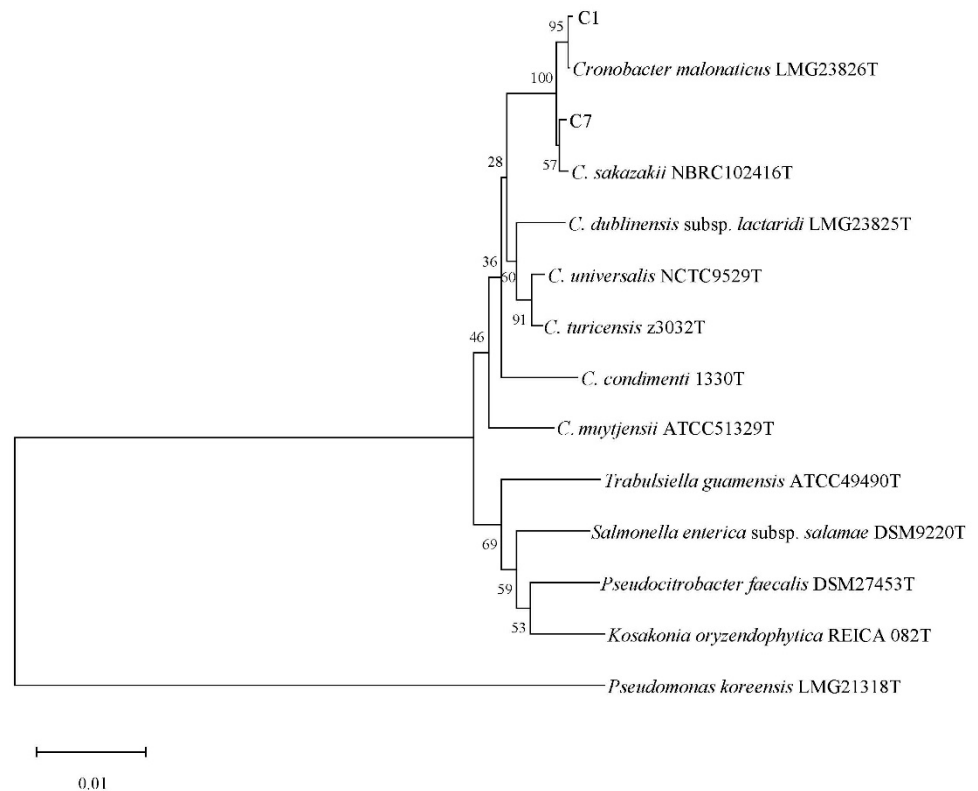


Figure S7. Phylogenetic trees of strain C1 and C7 based on their 16S rRNA gene sequences determined by the neighbor-joining method with the program package MEGA 11.0. Bootstrap confidence values were obtained using 2000 resamplings. Bar, 0.01 substitutions per site.

Table S1. Resistance of cucumber cultivars to CFW^a.

Cucumber cultivar	Disease incidence (%)		Disease index		Resistance
CL11	0.00 ± 0.00	a	0.00 ± 0.00	a	Resistant
EZZ	0.00 ± 0.00	a	0.00 ± 0.00	a	Resistant
JY409	16.67 ± 0.00	abc	8.32 ± 4.17	ab	Resistant
JY35	38.89 ± 9.62	c	23.61 ± 6.36	b	Moderately resistant
ZN106	33.34 ± 28.87	bc	15.27 ± 15.78	ab	Moderately resistant
ZN6	88.89 ± 19.24	d	51.39 ± 17.35	c	Susceptible

^a Different letters of the same column showed significant difference at $p < 0.05$ based on the Duncan's new multiple-range test

Table S2. Relative abundance of the predominant genera (>5.00%) in cucumber root.

Cultivar	Treatment	The relative abundance (%)									
		<i>Massilia</i>	Unclassified Oxalobacteraceae	Unclassified Rhodanobacteraceae	<i>Pseudomonas</i>	<i>Flavobacterium</i>	Unclassified Alcaligenaceae	<i>Shinella</i>	<i>Brevendimonas</i>	<i>Stenotrophomonas</i>	Unclassified Enterobacteriaceae
CL11	Mock	41.65	0.00	26.02	0.12	4.31	3.83	3.54	0.81	0.33	3.95
EZZ		47.83	0.75	21.9	0.28	1.06	7.31	4.1	1.26	0.69	0.00
JY409		28.08	0.13	13.08	13.86	6.56	5.89	3.83	1.56	1.66	5.67
JY35		39.71	0.92	15.40	0.15	4.28	9.33	7.56	1.24	0.64	0.13
ZN106		56.49	0.87	18.80	0.00	1.31	5.4	3.68	1.45	0.28	0.05
ZN6		49.77	1.12	20.03	0.53	0.66	5.06	4.15	1.7	0.72	0.16
CL11	<i>Foc</i> inoculation	61.55	0.12	10.09	0.11	1.47	4.75	4.89	0.57	1.02	3.67
EZZ		53.73	0.05	13.64	0.00	0.4	7.5	6.61	1.94	1.04	0.00
JY409		39.42	0.16	8.47	12.51	6.07	5.68	3.46	2.3	1.3	5.02
JY35		29.29	0.30	21.63	1.82	1.99	8.73	6.19	3.25	4.45	0.05
ZN106		42.57	0.36	5.18	1.25	24.75	5.83	4.87	2.58	0.94	0.88
ZN6		9.36	25.22	7.16	2.32	12.17	7.85	7.04	5.89	5.03	0.00

Table S3. Comparison of the relative abundance of *Massilia* and unclassified Oxalobacteraceae against the whole family Oxalobacteraceae in the cucumber root microbiota.

Cultivar	Treatment	The relative abundance (%)		
		Oxalobacteraceae	<i>Massilia</i> + unclassified Oxalobacteraceae	Proportions (%)
CL11	Mock	42.29	41.65	98.49
EZZ		48.88	48.58	99.39
JY409		28.20	28.21	100.04
JY35		40.63	40.63	100.00
ZN106		57.36	57.36	100.00
ZN6		50.98	50.89	99.82
CL11	<i>Foc</i> inoculation	61.80	61.67	99.79
EZZ		53.82	53.78	99.93
JY409		39.71	39.58	99.67
JY35		29.77	29.59	99.40
ZN106		42.92	42.93	100.02
ZN6		34.61	34.58	99.91

Table S4. Permutational multivariate analysis of variance (PERMANOVA) of the root bacterial microbiota of cucumber roots with (Group F) or without *Foc* (Group M) inoculation based on Bray-Curtis dissimilarity and 999 permutations.

Source	Df	Sums of Sq	Mean Sq	F Model	R ²	Pr (> F)
Group M						
Cultivar	5	0.86	0.17	1.25	0.34	0.156
Residuals	12	1.65	0.14		0.66	
Total	17	2.51			1	
Group F						
Cultivar	5	1.72	0.34	2.64	0.52	0.001
Residuals	12	1.56	0.13		0.48	
Total	17	3.28				
Groups M & F						
Cultivar	5	1.57	0.31	2.16	0.26	0.001
Inoculation	1	0.25	0.25	1.75	0.04	0.040
Residuals	29	4.22	0.15		0.70	
Total	35	6.04			1.00	

Table S5. Analysis of similarity (ANOSIM) of the cucumber cultivars with mock (M) or *Foc* inoculation (F).

Paired groups	R value	<i>p</i> value
CL11M vs. CL11F	0.44	0.10
EZZM vs. EZZF	0.04	0.51
JY409M vs. JY409F	−0.04	0.62
JY35M vs. JY35F	0.11	0.49
ZN106M vs. ZN106F	0.52	0.10
ZN6M vs. ZN6F	0.41	0.19

Table S6. Differential bacterial ASVs across cucumber cultivars with mock or *Foc* inoculation ^a.

Treatment	Differential ASV	Genus	Proportions (%)								<i>p</i> value				
			CL11		EZZ		JY409		JY35			ZN106		ZN6	
Mock inocula- tion	ASV162	<i>Cronobacter</i>	1.75 ± 3.03 a		0 ± 0 a		0 ± 0 a		0 ± 0 a		1.25 ± 0.34 b		0 ± 0 a		0.03
	ASV103	<i>Pseudomonas</i>	0 ± 0 a		0 ± 0 a		1.87 ± 0.36 b		0 ± 0 a		0 ± 0 a		0 ± 0 a		0.00
	ASV124	<i>Noviherbaspirillum</i>	0.65 ± 0.46 a		0.24 ± 0.41 ab		0 ± 0 b		0 ± 0 b		0 ± 0 b		0.09 ± 0.15 b		0.05
	ASV74	<i>Massilia</i>	0.02 ± 0.03 a		0 ± 0 a		0 ± 0 a		0 ± 0 a		0 ± 0 a		0.15 ± 0.05 b		0.01
<i>Foc</i> inocula- tion	ASV1	<i>Massilia</i>	52.55 ± 7.44 a		41.76 ± 11.21 ab		32.96 ± 8.94 bc		23.12 ± 3.15 c		34.61 ± 12.93 bc		5.36 ± 9.29 d		0.03
	ASV95	<i>Flavobacterium</i>	1.25 ± 0.94 a		0.074 ± 0.13 a		5.79 ± 4.19 a		0.66 ± 1.14 a		23.79 ± 13.80 b		0.10 ± 0.17 a		0.03
	ASV4	<i>Massilia</i>	6.24 ± 2.82 a		6.41 ± 2.03 a		3.84 ± 0.31 ab		2.75 ± 0.36 bc		4.55 ± 0.94 ab		0.55 ± 0.95 c		0.02
	ASV48	<i>Pseudomonas</i>	0.11 ± 0.20 a		0 ± 0 a		10.98 ± 1.02 b		1.44 ± 1.40 ac		1.11 ± 0.96 ac		1.97 ± 1.03 c		0.03
	ASV103	<i>Pseudomonas</i>	0 ± 0 a		0 ± 0 a		1.53 ± 0.56 b		0 ± 0 a		0.14 ± 0.24 a		0.16 ± 0.27 a		0.03
	ASV33	Unclassified_f_ Rhodanobacteraceae	0.02 ± 0.03 a		0.11 ± 0.04 b		0 ± 0 a		0 ± 0 a		0 ± 0 a		0 ± 0 a		0.01

^aData were analyzed by the Kruskal-Wallis H test and verified by FDR and Tukey-Kramer with the confidence at 0.95; different letters of the same row showed significant difference at $p < 0.05$.

Table S7. Isolation and identification of the culturable bacteria isolated from cucumber roots by using the conventional plating method.

Cucumber cultivar	Isolates	Inhibitory rate (%)	Taxa based on 16S rRNA gene sequences	Taxa and ASVs based on Illumina high-throughput analysis		Homology (%)
				Genus	ASV	
CL11	P25, P26, P28, P29	33.17 ± 2.93 ~ 37.77 ± 2.60	<i>Pantoea</i>	Unclassified_o__Enterobacterales	ASV410	93.1–97.6
	D34, D37, D39	17.78 ± 2.13 ~ 19.10 ± 1.60	<i>Delftia</i>	<i>Delftia</i>	ASV104	100.0
	S36	22.36 ± 2.36	<i>Sphingomonas</i>	<i>Sphingomonas</i>	ASV329	98.7
	D38	23.50 ± 5.32	<i>Roseomonas</i>	<i>Devosia</i>	ASV497	87.4
	S40	25.91 ± 3.45	<i>Sphingomonas</i>	<i>Sphingomonas</i>	ASV19	100.0
	S41	29.82 ± 3.29	<i>Hephaestia</i>	<i>Hephaestia</i>	ASV548	99.4
	S42	22.90 ± 2.78	<i>Sphingomonas</i>	<i>Sphingomonas</i>	ASV375	97.4
JY409	D43	28.82 ± 3.01	<i>Roseomonas</i>	<i>Devosia</i>	ASV497	87.4
	Sh44	13.34 ± 1.13	<i>Shinella</i>	<i>Shinella</i>	ASV40	100.0
	Bo45	18.12 ± 2.65	<i>Bosea</i>	<i>Bosea</i>	ASV290	100.0
	Ps213	21.63 ± 4.41	<i>Pseudomonas</i>	<i>Pseudomonas</i>	ASV466	99.7
ZN6	Ps15-Ps19	22.37 ± 2.61 ~ 25.20 ± 3.71	<i>Pseudomonas</i>	<i>Pseudomonas</i>	ASV466	99.7
	Pa30	17.33 ± 5.01	<i>Roseomonas</i>	<i>Devosia</i>	ASV497	87.4
	H31	24.21 ± 3.28	<i>Sphingomonas</i>	Unclassified_f__Sphingomonadaceae	ASV105	100.0
	D32	20.34 ± 1.22	<i>Delftia</i>	<i>Delftia</i>	ASV104	100.0