Supplementary Information

to

Antimicrobial resistant bacteria in wastewater-irrigated Mexican soils and transfer of resistant bacteria from irrigated soils to cilantro plants

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Table S1. Physiochemical wastewater parameters and concentrations of antibiotic and biocidal compound present in or spiked to irrigation water types (according to Soufi *et al.,* 2025)

Parameters	WWTP influent	WWTP effluent	Spiked concentrations
pH	7.4	7.4	
EC	1300	1300	
BOD [mg/L]	50	12	
COD [mg/L]	320	110	
TOC [mg/L]	65	34	
Azithromycin [ng/L]	0-1390	0-1330	695000
Ciprofloxacin [ng/L]	2350-3390	1940-2270	1695000
Clindamycin [ng/L]	< DL	< DL	60000***
(Anhydro)Erythromycin [ng/L]*	210 (<QL) -370	170-250 (<QL)	185000
Sulfamethoxazole [ng/L]	3190-3990	2820-3780	1995000
Trimethoprim [ng/L]	1100-1300	1200-1400	650000
Sum ATMACs [ng/L]	170	98	84546
ATMAC-C8	12	12	5770
ATMAC-C10	11	10	5326
ATMAC-C12	24	16	11766
ATMAC-C14	13	11	6493
ATMAC-C16	110	49	55191
Sum BACs [ng/L]	524	200	261650
BAC-C8	9	9	4322
BAC-C10	12	12	6115
BAC-C12	318	95	158921
BAC-C14	141	53	70559
BAC-C16	19	14	9287
BAC-C18	25	17	12446
Sum DADMACs [ng/L]	208	53	156452
DADMAC-C8	0 (21)**	0 (21)**	10527
DADMAC-C10	0 (21)**	0 (21)**	10527
DADMAC-C12	0 (21)**	0 (21)**	10527
DADMAC-C14	0 (21)**	0 (21)**	10527
DADMAC-C16	0 (21)**	0 (21)**	10527
DADMAC-C18	208	53	103817

*Anhydroerythromycin = degradation product of erythromycin

** concentration <DL, therefore the median of all QAACs was used as concentration

*** Clindamycin was not detected. The spiked concentration was calculated using the concentration found in Siemens *et al.,* (2008) (120 [ng/L]).

EC: electrical conductivity; BOD: biochemical oxygen demand; COD: chemical oxygen demand; TOC: total organic carbon; N_{tot}: total nitrogen; NO_3^--N: Nitrate content; NH_4^+-N: ammonium content; ATMACs: alkyltrimethyl ammonium compounds; BACs: benzylalkyldimethyl ammonium compounds; DADMAC: dialkyldimethyl ammonium compounds, QL: quantification limit; DL: detection limit

Table S2 Details of primer systems used in this study.

Target	Primers	5'-3' sequences	Primer concentration (μM)	Annealing temperature	Product size	Reference
Repetitive extragenic palindromic (REP) sequence	BOXA1R	CTACGGCAAGGCGACGCTGACG	1 μM	53°C	Strain specific genomic fingerprints	Versalovic et al. (1994) Glaeser et al. (2013)
16S rRNA gene	EUB9F EUB1492R	GAGTTTGATCMTGGCTCAG CGGTTACCTTGTTACGACTT	0.2 μM 0.2 μM	54°C	~1470 bp	Lane (1991)
nrdA	nrdA-F nrdA-R	GAACTGGATTCCCGACCTGTTC TTCGATTTGACGTACAAGTTCTGG	0.2 μM 0.2 μM	56°C	954 bp	Spilker et al. (2012)
gyrA	gyrA-F1 gyrA-R1	CCGGTATCGCTGGAAGAAGAGA CCTGCTCGCTGCCGTCGTA	0.2 μM 0.2 μM	57°C	436 bp	Magallon et al. (2021)
parC	parC-F parC-R	ATCGGCGACGGCCTGAAGCC CGGGATTCGGTATAACGCAT	0.2 μM	55°C	273 bp	Furlan et al. (2018)
qnrB	qnrBm-F qnrBm-R	GGMATHGAAATTCGCCACTG TTTGCYGYYCGCCAGTCGAA	0.2 μM 0.2 μM	54°C	264 bp	Cattoir et al. (2007); Alipour et al. (2024)
qnrS	qnrSm-F qnrSm-R	GCAAGTTCATTGAACAGGGT TCTAAACCGTCGAGTTCGGCG	0.2 μM 0.2 μM	54°C	428 bp	Cattoir et al. (2007); Alipour et al. (2024)

Table S3. Absolute abundance (log_{10} CFU mL^{-1}) of bacteria cultivated on MH and R2A from wastewater used for incubation experiment. The data represented the mean CFU mL^{-1} obtained after spotting four independent dilution series of each wastewater types (influent, effluent, and both spiked) on respective media plates (four technical replications, see Fig. S2). Standard deviations reflect variability among the four technical replicates (independent dilution series; n=4). For water samples technical replicates were compared because just one water sample per water sample type was available for the experiment. Asterisk (*) showed significant difference (Two-way ANOVA; $p<0.05$).

Targeted heterotrophic culturable bacteria	Concentration in log_{10} CFU mL^{-1} [Mean (±Standard deviation)]			
	Unspiked		Spiked	
	Influent	Effluent	Influent	Effluent
Bacteria cultivated on MH (-/+ supplements) 37°C, 24 h				
Bacteria on MH	5.9 (± 5.1)	5.3 (± 4.6)*	6.6 (± 6.0)*	5.6 (± 0.0)*
CIP resistant bacteria (MH+CIP)	4.4 (± 4.1)	4.1 (± 3.5)	4.2 (± 3.8)	3.4 (± 3.2)*
TRI/SUL resistant bacteria (MH+TRI/SUL)	5.3 (± 4.3)	3.7 (± 3.3)*	5.3 (± 0.0)	5.1 (± 4.7)*
ERY/CLI resistant bacteria (MH+ERY/CLI)	5.4 (± 4.7)	3.9 (± 3.5)*	6.5 (± 6.1)*	4.9 (± 4.6)*
BAC-C12 tolerant bacteria (MH+BAC-C12)	4.0 (± 3.3)	3.3 (± 2.9)*	4.3 (± 3.6)*	3.6 (± 3.0)*
Bacteria cultivated on R2A (-/+ supplements) 25°C, 48 h				
Bacteria on R2A	6.9 (± 6.2)	6.0 (± 5.3)*	6.9 (± 6.2)	6.2 (± 5.4)
CIP resistant bacteria (R2A+CIP)	5.7 (± 5.4)	4.2 (± 3.8)*	5.9 (± 5.3)	5.6 (± 5.2)*
TRI/SUL resistant bacteria (R2A+TRI/SUL)	5.6 (± 5.4)	4.4 (± 3.4)*	6.1 (± 5.7)*	5.3 (± 4.9)*
ERY/CLI resistant bacteria (R2A+ERY/CLI)	6.3 (± 5.6)	5.0 (± 5.0)*	6.7 (± 5.9)*	5.8 (± 5.0)*
BAC-C12 tolerant bacteria (R2A+BAC-C12)	3.6 (± 3.0)	3.3 (± 2.4)*	4.7 (± 0.0)*	4.0 (± 3.6)*
Significance test to		to Influent	to Unspiked-Influent	to Unspiked-Effluent

Table S4. Absolute abundance (log_{10} CFU g^{-1}) of bacteria cultivated on MH and R2A from soils before irrigation (0 days) and after four weeks following irrigation with unspiked-influent, unspiked-effluent, spiked-influent, and spiked-effluent, respectively. The data represent the mean CFU g^{-1} obtained for four fields analysed per soil type (Leptosol, Phaeozem, and Vertisol). Standard deviations given in brackets reflect variability among the four fields analysed per soil type (=biological replicates). Significant differences are marked in bold ($p<0.05$, One-way ANOVA). The values for each biological replicate are arithmetic means of four technical replications (independent dilution series). For details see Figure S2.

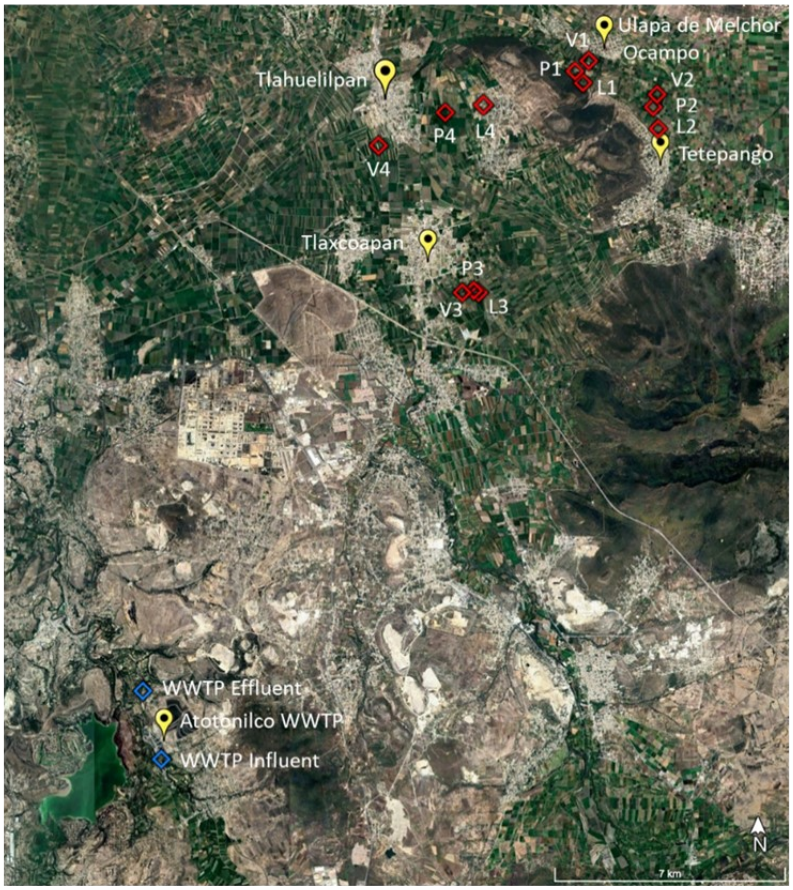
	Soils - 0 days (before water addition)			**Soils - four weeks after water addition**											
Targeted cultivable heterotrophic bacteria	**Absolute abundance in log_{10} CFU g^{-1} [Mean (±Standard deviation)]**														
Soil types	**Leptosol**	**Phaeozem**	**Vertisol**	**Leptosol**				**Phaeozem**				**Vertisol**			
				Unspiked		Spiked		Unspiked		Spiked		Unspiked		Spiked	
				Influent	Effluent	Influent	Effluent	Influent	Effluent	Influent	Effluent	Influent	Effluent	Influent	Effluent
Bacteria cultivated on MH (-/+ supplements) 37°C, 24 h															
Bacteria on MH	**7.1 (± 0.1)**	6.8 (± 0.1)	**6.4 (± 0.6)**	6.6 (± 0.3)	6.6 (± 0.2)	6.7 (± 0.3)	6.6 (± 0.2)	6.5 (± 0.4)	6.1 (± 0.4)	6.4 (± 0.3)	6.3 (± 0.4)	6.7 (± 0.3)	6.7 (± 0.4)	6.4 (± 0.6)	6.8 (± 0.3)
CIP resistant bacteria (MH+CIP)	5.7 (± 0.3)	5.6 (± 0.4)	5.7 (± 0.2)	5.1 (± 0.6)	5.1 (±0.6)	5.0 (± 0.7)	5.2 (± 0.6)	4.8 (± 0.4)	5.1 (± 0.9)	5.1 (± 0.8)	5.4 (± 1.0)	5.0 (± 0.7)	5.1 (± 0.7)	5.2 (± 0.8)	5.1 (± 0.8)
TRI/SUL resistant bacteria (MH+TRI/SUL)	5.4 (± 0.2)	5.3 (± 0.1)	5.3 (± 0.3)	5.9 (± 0.1)	5.6 (± 0.4)	5.7 (± 0.3)	6.1 (± 0.2)	5.6 (± 0.6)	5.5 (± 0.5)	5.6 (± 0.4)	5.6 (± 0.6)	5.5 (± 0.5)	5.8 (± 0.2)	5.9 (± 0.1)	5.8 (± 0.1)
ERY/CLI resistant bacteria (MH+ERY/CLI)	5.5 (± 0.4)	4.9 (± 0.2)	4.9 (± 0.3)	5.6 (±0.5)	5.7 (± 0.2)	5.3 (± 0.2)	5.5 (± 0.3)	5.2 (± 0.6)	5.1 (± 0.6)	5.0 (± 0.2)	5.2 (± 0.6)	5.5 (± 0.4)	5.2 (± 0.6)	5.8 (± 0.2)	5.6 (± 0.2)
BAC-C12 tolerant bacteria (MH+BAC-C12)	3.5 (± 0.6)	3.7 (± 0.5)	4.0 (± 0.2)	4.2 (±0.1)	4.2 (± 0.2)	4.5 (± 0.7)	4.3 (± 0.1)	4.2 (± 0.5)	4.3 (± 0.6)	4.3 (± 0.5)	4.3 (± 0.4)	4.7 (± 0.4)	4.7 (± 0.6)	4.7 (± 0.4)	4.5 (± 0.4)
Bacteria cultivated on R2A (-/+ supplements) 25°C, 48 h															
Bacteria on R2A	6.6 (± 0.3)	6.4 (± 0.5)	6.5 (± 0.4)	6.7 (± 0.5)	6.7 (± 0.5)	6.6 (± 0.5)	6.5 (± 0.5)	6.4 (± 0.4)	6.1 (± 0.6)	6.2 (± 0.4)	6.3 (± 0.2)	6.3 (± 0.7)	6.5 (± 0.7)	6.3 (± 0.6)	6.4 (± 0.6)
CIP resistant bacteria (R2A+CIP)	6.0 (± 0.5)	5.7 (± 0.3)	5.4 (± 0.5)	5.8 (± 1.2)	6.0 (± 1.0)	6.0 (± 1.2)	5.8 (± 1.0)	5.6 (± 0.8)	5.5 (± 0.9)	5.6 (± 0.7)	5.6 (± 0.9)	5.9 (± 0.8)	5.8 (± 0.9)	5.9 (± 0.9)	5.5 (± 1.0)
TRI/SUL resistant bacteria (R2A+TRI/SUL)	5.0 (± 0.1)	5.1 (± 0.1)	4.9 (± 0.2)	5.1 (± 0.5)	5.1 (± 0.4)	5.0 (± 0.5)	5.0 (± 0.3)	4.7 (± 0.7)	4.7 (± 0.8)	4.7 (± 0.6)	4.7 (± 0.6)	5.0 (± 0.3)	5.3 (± 0.6)	4.7 (± 0.4)	5.5 (± 0.6)
ERY/CLI resistant bacteria (R2A+ERY/CLI)	6.1 (± 0.6)	5.5 (± 0.5)	5.7 (± 0.6)	6.0 (± 0.5)	5.8 (± 0.5)	5.6 (± 0.5)	5.8 (± 0.6)	5.5 (± 0.5)	5.2 (± 0.7)	5.5 (± 0.5)	5.5 (± 0.8)	5.7 (± 0.7)	5.7 (± 0.5)	5.7 (± 0.6)	5.9 (± 0.3)
BAC-C12 tolerant bacteria (R2A+BAC-C12)	3.6 (± 0.4)	3.7 (± 0.4)	4.0 (± 0.1)	4.0 (± 0.4)	4.0 (± 0.4)	4.2 (± 0.9)	4.0 (± 0.5)	4.0 (± 0.4)	4.0 (± 0.5)	4.0 (± 0.7)	4.0 (± 0.5)	4.3 (± 0.3)	4.8 (± 0.9)	4.1 (± 0.3)	4.4 (± 0.7)

Table S5: Phylogenetic overview of bacterial strains cultivated on MH/R2A plates containing CIP from leaves and roots of cilantro grown in soil types (Leptosol, Phaeozem, Vertisol) irrigated with unspiked-influent, unspiked-effluent, spiked-influent, and spiked-effluent wastewater. Similarity values represented ranges of pairwise sequence similarities of phylotype strains to next related type strain placed within a phylotype cluster. Number inside the bracket represented the number of strains sharing identical DNA fingerprint pattern (BOX-PCR).

Cilantro tissue			Cilantro leaves												Cilantro roots											
Soil types			Leptosol				Phaeozem				Vertisol				Leptosol				Phaeozem				Vertisol			
Spiking of wastewaters			Unspiked		Spiked		Unspiked		Spiked		Unspiked		Spiked		Unspiked		Spiked		Unspiked		Spiked		Unspiked		Spiked	
Genus	Phylotype	Pairwise 16S rRNA gene similarity to type strains of given species	Influent	Effluent	Influent	Effluent	Influent	Effluent	Influent	Effluent	Influent	Effluent	Influent	Effluent	Influent	Effluent	Influent	Effluent	Influent	Effluent	Influent	Effluent	Influent	Effluent	Influent	Effluent
Achromobacter	1	99–100%, *A. spanius/A. marplatensis/A. deleyi/A. kerstersii/A. piechaudii*		6 (4)	2 (1)			7 (5)	6 (4)	1		9 (7)	1		2	3	10 (2)			8 (1)	3	3	6 (1)	3	7 (2)	
Bosea	2	98.8%, B. lathyri										1														
	1	99.2-99.4%, *B. robiniae/B. thiooxidans/B. spartocytisi*										1														
Cellulomonas	1	99.1%, *C. pakistanensis*											1													
Cellulosimicrobium	1	98.5-99.7%, *C. funkei*		3 (2)	4 (3)			1				2 (1)	2 (2)											1		
Lysinibacillus	1	99.5-100%, *L. fusiformis*								5 (4)						1	1									
	2	99.5%, *L. xylanilyticus*										2(1)														
	3	99.8-100%, *L. capsici*						2																		
Microbacterium	1	99.3%, *M. lacus*										1														
Paenarthrobacter	1	99.2-100%, *P. nicotinovorans*								2 (1)						3 (1)	2			1			1	1		
	2	98.6-100%, *P. aurescens/P. nitroguajacolicus*		1	2 (2)						2	2 (1)			2	1				3	1			2 (1)		
Paenibacillus	1	98.2-100%, *P. etheri*		1	1										1											
	2	97%, *P. auburnensis/P. typhae*								1	1		2													
	3	99.4-99.5%, *P. taohuashanense*								1											1					
	4	99.5%, *P. cucumis/P. polysaccharolyticus*							1																	
	5	99.4-99.7%, *P. xylanexedens/P. amylolyticus*								1																
	6	99.2-99.6%, *P. ottowii/P. brasilensis*									11 (8)	1														
Pseudarthrobacter	1	99.2%, *P. defluvii/P. niigatensis*										1														
Pseudomonas	2	100%, *P. atacamensis/P.*								2																

		iranenesis/P. koreensis								(1)																
	1	99.7%, *P. citrulli*											1													
Stenotrophomonas	1	99.6-100%, *S. lactitubi*			2 (1)																**1**		1		1	
	2	99.6-100%, *S. rhizophila/S. nematodicola*	1 (1)	2 (1)					2	2	1		1 (1)			2 (1)					2 (1)	1				
Stutzerimonas	1	99-100%, *S. chloritidismutans/S. kunmingensis*	6 (4)												1											
Arthrobacter	1	97.6%, *A. crystallopoietes*																		1						
Oerskovia	1	99.9-100%, *O. jenensis*																		1				1		
		Total strains	**108**												**79**											

Supplementary Figures



Location	Years of UWW irrigation	Soil type	Coordinates	
Ulapa de Melchor Ocampo	111	Leptosol (L1)	20° 8' 9.5" N	99° 10' 31.3" W
		Phaeozem (P1)	20° 8' 13.6" N	99° 10' 23.5" W
		Vertisol (V1)	20° 8' 30.9" N	99° 10' 25.3" W
Between Tetepango and Ulapa	60	Leptosol (L2)	20° 7' 24.5" N	99° 9' 13.3" W
		Phaeozem (P2)	20° 7' 45.7" N	99° 9' 18.4" W
		Vertisol (V2)	20° 7' 58" N	99° 9' 14.3" W
Tlaxcoapan (Bojayito Chico)	92	Leptosol (L3)	20° 4' 44.9" N	99° 12' 20.1" W
		Phaeozem (P3)	20° 4' 47" N	99° 12' 25" W
		Vertisol (V3)	20° 4' 45" N	99° 12' 37" W
Tlahuelilpan	101	Leptosol (L4)	20° 7' 28.4" N	99° 12' 36.3" W
		Phaeozem (P4)	20° 7' 40.4" N	99° 12' 54.5" W
		Vertisol (V4)	20° 7' 8.24" N	99° 14' 3.78" W

Figure S1. Geographical area, location, coordinates, and years of untreated wastewater (UWW) irrigation of the agricultural fields used for the study (L1: Leptosol field 1; L2: Leptosol Field 2; L3: Leptosol Field 3; L4: Leptosol Field 4; P1: Phaeozem field 1; P2: Phaeozem Field 2; P3: Phaeozem Field 3; P4: Phaeozem Field4; V1: Vertisol field 1; V2: Vertisol Field 2; V3: Vertisol Field 3; V4: Vertisol Field 4). Adapted from Soufi *et al.,* (2025)

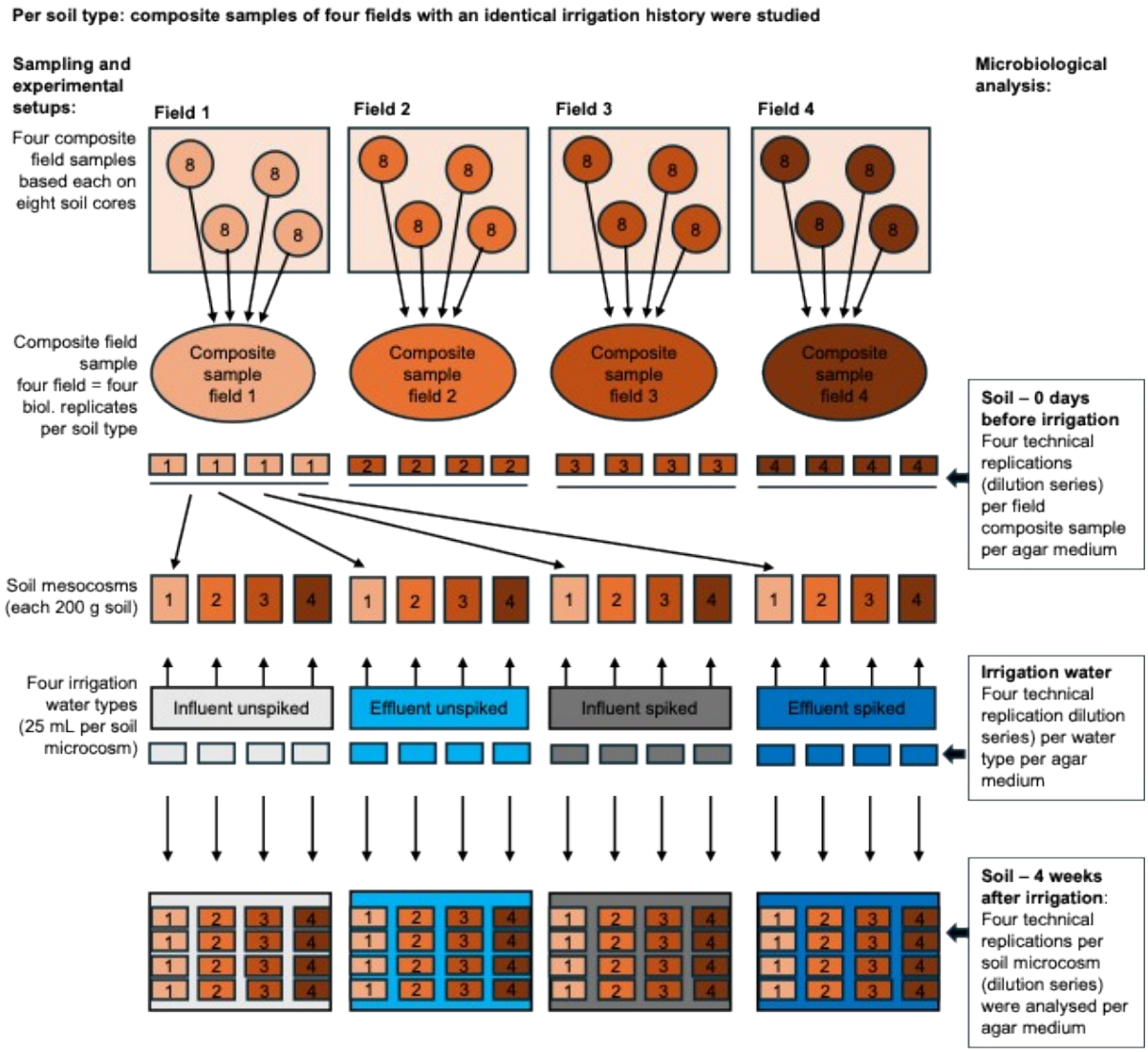


Figure S2 Overview of sample collection in Mexican fields, set up of the incubation experiment, and performed microbiological analyses

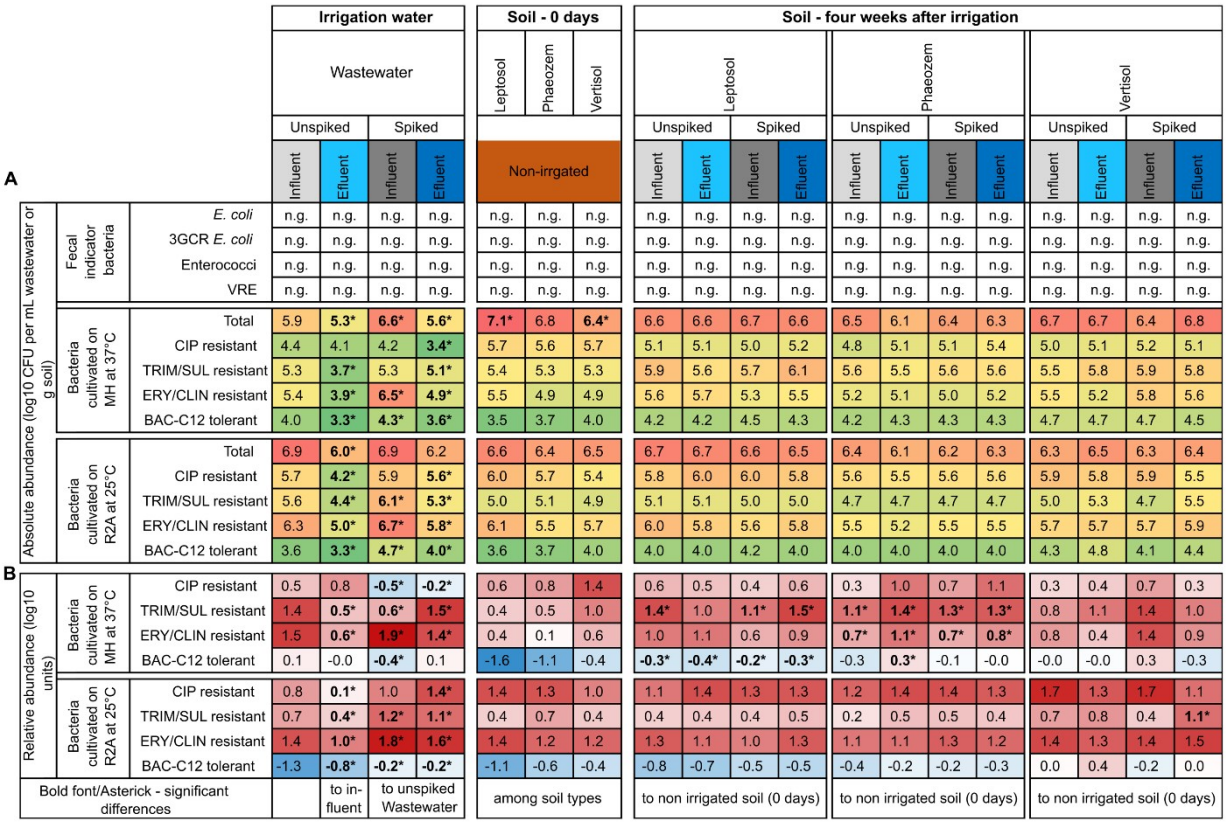


Figure S3. Heat map showing **(A)** mean absolute abundance (Log10 CFU mL^{-1} or g^{-1}) of total and antimicrobial (CIP, TRIM/SUL, ERY/CLIN, and BAC-C12) resistant bacteria cultivable on MH and R2A and **(B)** mean relative abundances in WWTP influent and effluent and soil before (0 days) and after irrigation with both unspiked and spiked influent or effluent. Significant differences (p<0.05) are indicated with bold font and asterisks (*). No growth: n.g. Mean values of four technical replications (irrigation water types) or four biological replications (four fields per soil type) are presented. Each biological replicate based on four technical replications. For details see Figure S2.

A


85 WW-T-S-D-MHB100-2, PQ753580
85 WW-U-U-D-MHSUL-1, PQ753613
Pseudomonas kielensis MBT-1T, MW377589
WW-U-U-D-MHSUL-3, PQ753615
Pseudomonas crudilactis UCMA 17988T, MH016575
Pseudomonas arsenicoxydans VC-1T, FN645213
Pseudomonas kilonensis 520-20T, AJ292426
98 WW-T-U-D-MH-4, PQ753550
98 WW-T-S-D-MHB50-1, PQ753565
Pseudomonas morbosilactucae MAFF 302030T, LC659974
Pseudomonas bubulae TH39T, KX186949
Pseudomonas endophytica BSTT44T, LN624760
WW-U-U-D-MHCIP4-4, PQ753599
91 WW-U-U-D-MHB100-1, PQ753581
Pseudomonas helleri DSM 29165T, KP738715
76 Pseudomonas siliginis SWRI31T, JAHSTW000000000
Pseudomonas triticicola SWRI88T, JAHSTX000000000
77 Pseudomonas baetica a390T, FM201274
Pseudomonas migulae CIP 105470T, AF074383
Pseudomonas spelaei SJ/9/1T, HQ844525
Pseudomonas karstica HJ/4T, HQ844524
Pseudomonas zeae OE 48.2T, CP077090
78 WW-U-U-D-MHB100-4, PQ753584
78 Pseudomonas veronii CIP 104663T, AF064460
87 WW-U-U-D-MHB100-3, PQ753583
99 Pseudomonas extremaustralis CT14-3T, AJ583501
88 Pseudomonas petroselini MAFF 311094T, LC647547
Pseudomonas grimontii CFML 97-514T, AF268029
97 Pseudomonas brenneri CFML 97-391T, AF268968
Pseudomonas proteolytica CMS 64T, AJ537603
74 WW-T-U-D-MHB100-3, PQ753577
PLBPW-PTST1-MHCIP1-2, PV243484
Pseudomonas atacamensis M7D1T, SSBS01000000
95 Pseudomonas iranensis SWRI54T, CP077092
Pseudomonas koreensis Ps 9-14T, AF468452
Pseudomonas glycinae MS586T, MG692779
Pseudomonas granadensis F-278,770T, HG764746
88 WW-T-U-D-MHSUL-3, PQ753607
Pseudomonas vancouverensis DhA-51T, AJ011507
WW-T-U-D-MHB50-1, PQ753561
Pseudomonas citri OPS13-3T, OM327744
PLBPW-VUST3-MHCIP1-1A, PV243455
"Pseudomonas citrulli" K18T, OR725083
82 WW-T-U-D-MHB100-4, PQ753578
WW-T-U-D-MHB50-2, PQ753562
WW-T-U-D-MHB50-4, PQ753564
WW-T-U-D-MHB100-1, PQ753575
WW-T-S-D-MHB50-3, PQ753567
WW-T-U-D-MHB100-2, PQ753576
WW-T-S-D-MHB100-1, PQ753579
WW-U-U-D-MHB100-2, PQ753582
86 Pseudomonas shahriarae SWRI52T, CP077085
87 Pseudomonas gessardii CIP 105469T, AF074384
88 WW-U-U-D-MHB50-2, PQ753569
Pseudomonas carnis Speck CT, KX186970
89 WW-U-U-D-MH-2, PQ753555
WW-T-S-D-MHCIP4-1, PQ753593
89 WW-U-U-D-MHSUL-2, PQ753614
Pseudomonas pharmacofabricae ZYSR67-Z sourceT, KX910087
92 Pseudomonas fluvialis ASS-1T, KU248754
Pseudomonas mangrovi TC11T, MH137036
Pseudomonas knackmussii B13, AF039489
Pseudomonas aeruginosa DSM 50071T, HE978271
WW-T-U-D-MHCIP4-1, PQ753589
Pseudomonas peli R-20805T, AM114534
Pseudomonas guineae LMG 24016T, AM491810
Pseudomonas flexibilis ATCC 29606T, GU269546
PLBPW-LUUS2-MHCIP1-1, PV243494
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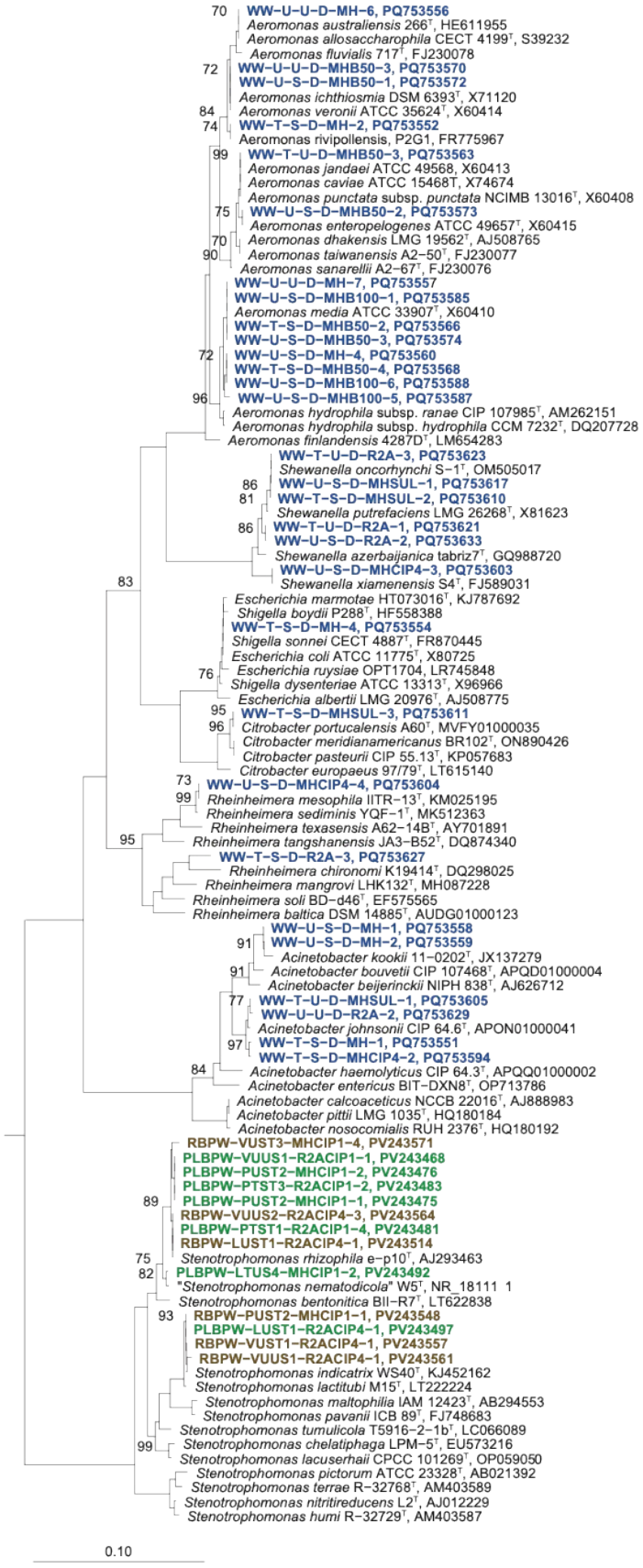
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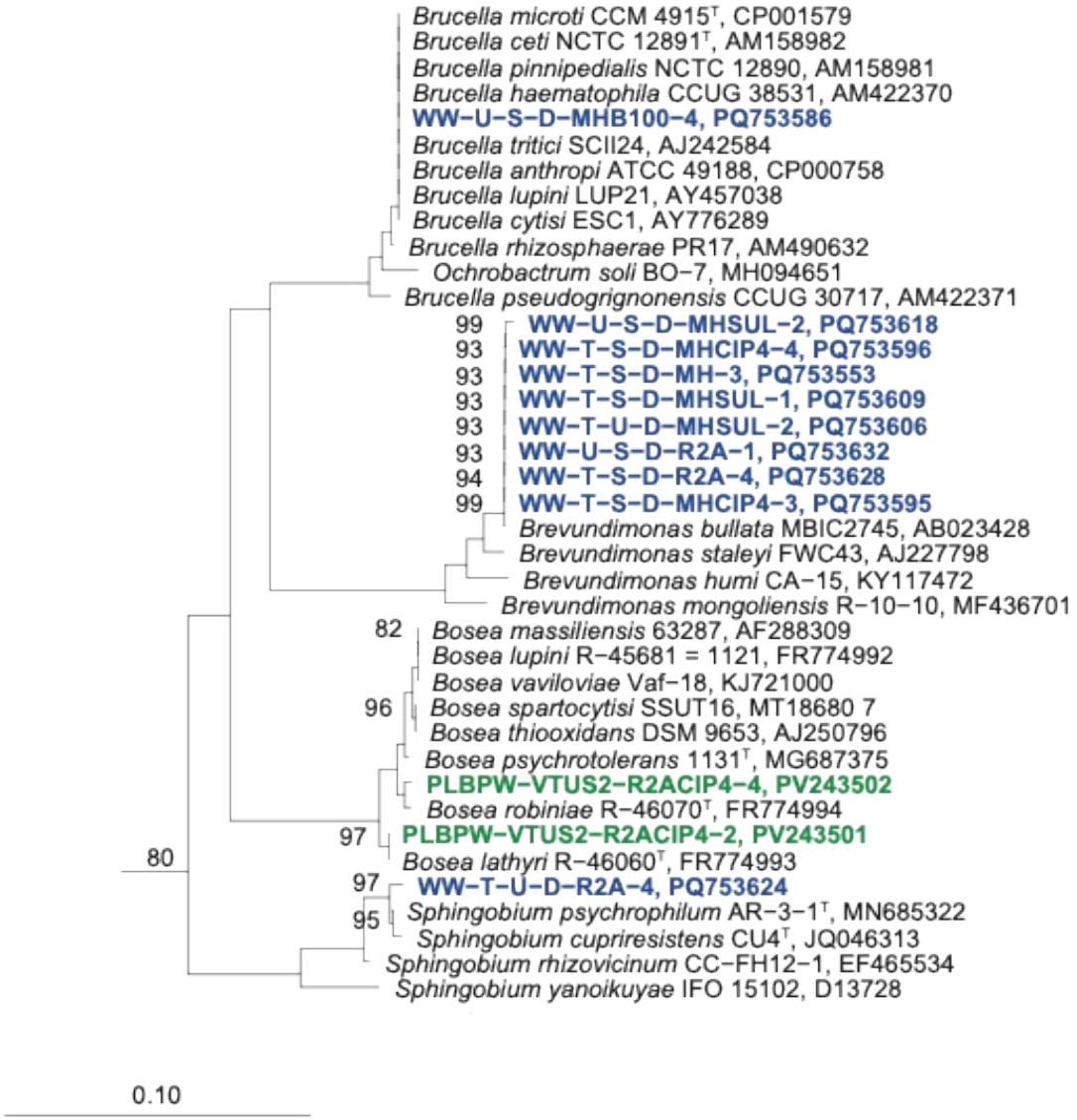
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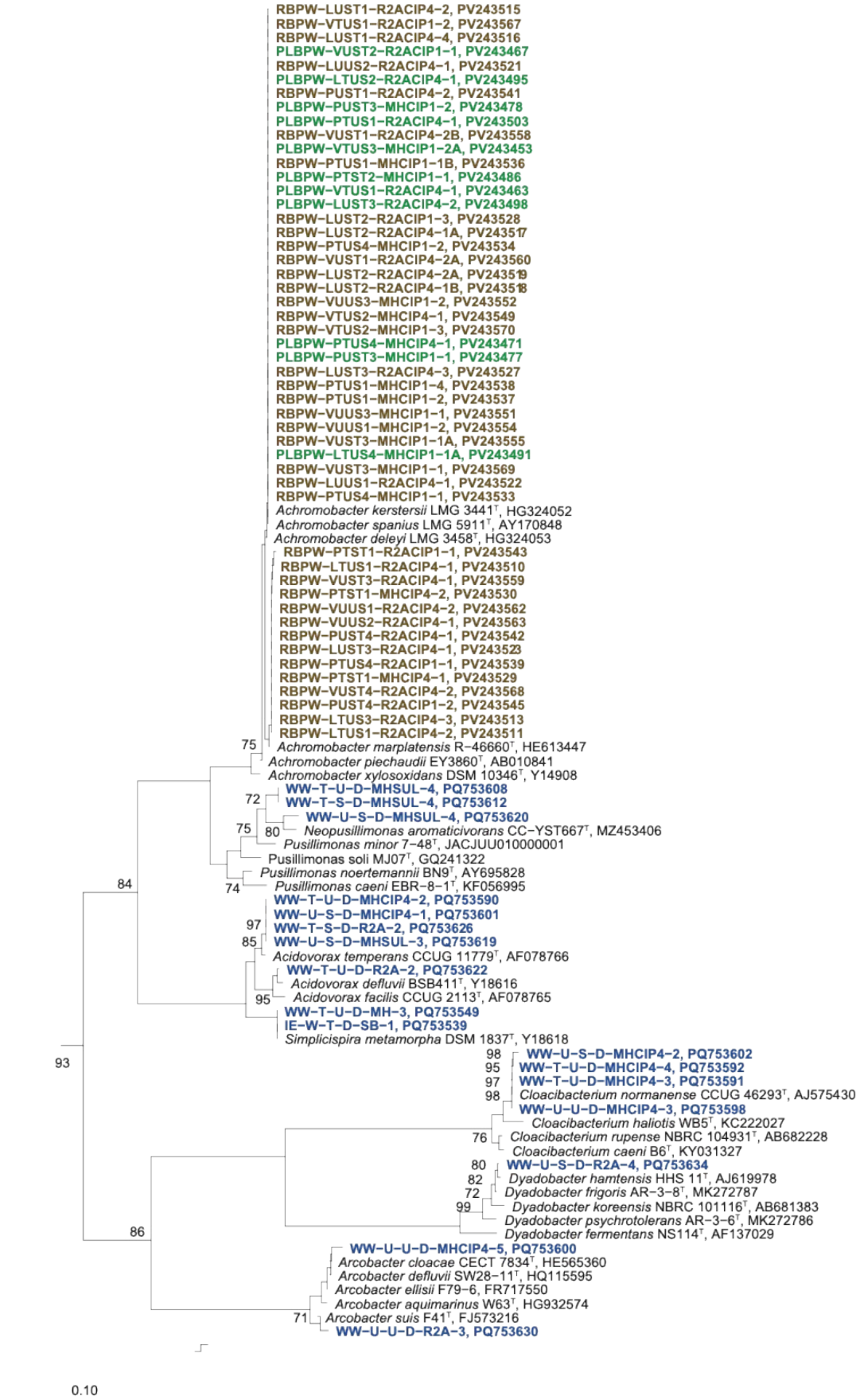
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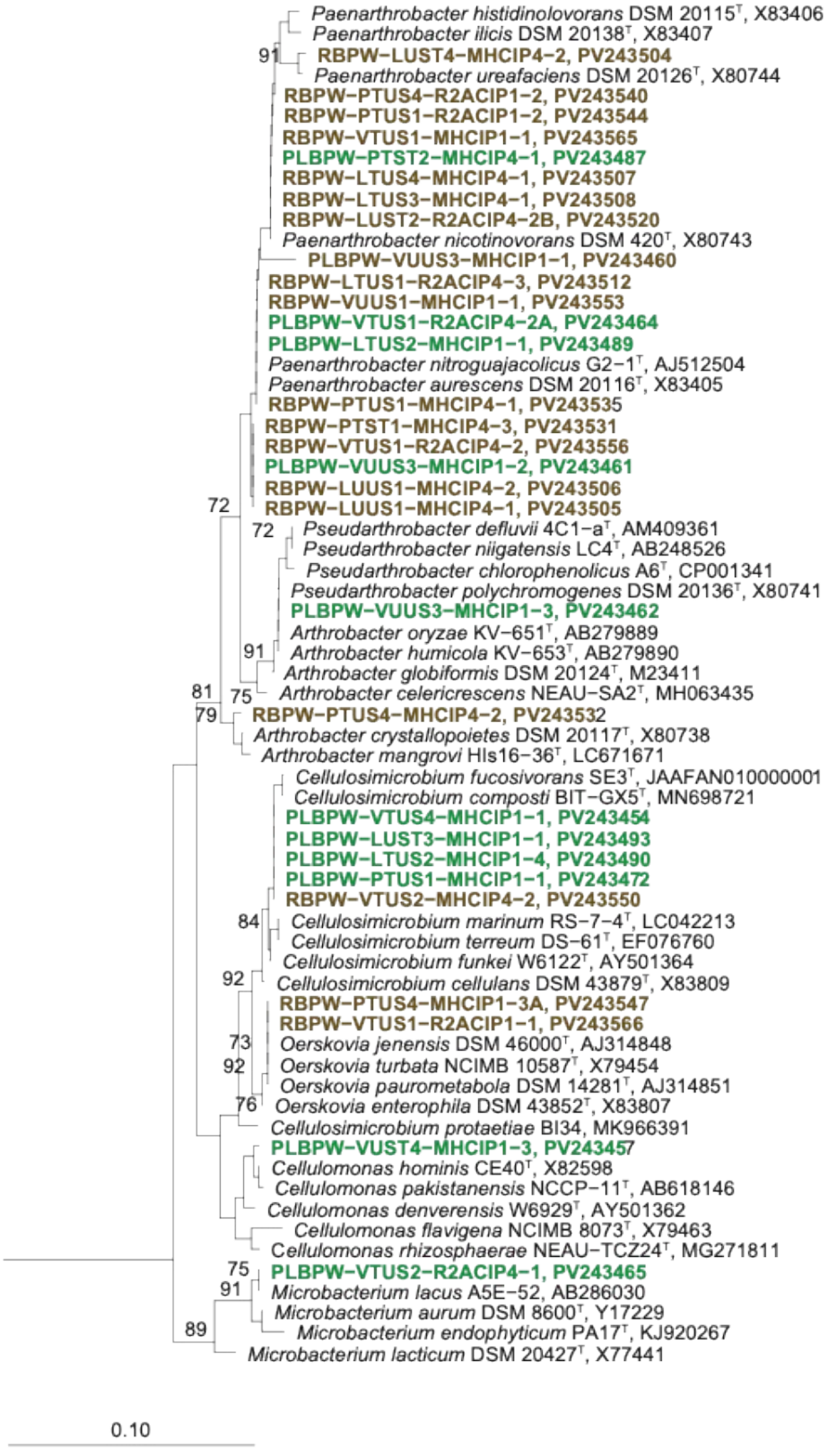
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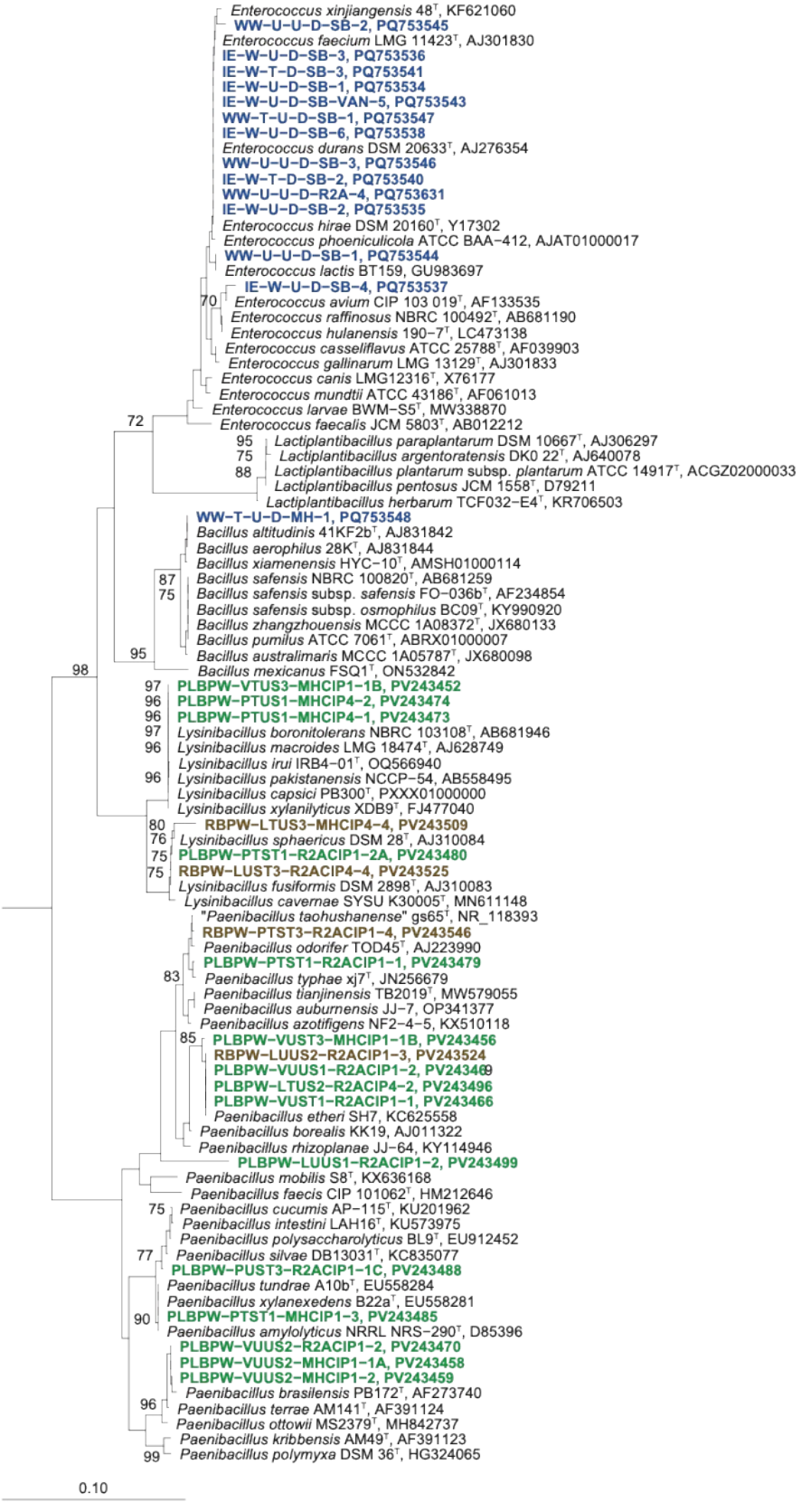
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Lysinibacillus macroides LMG 18474T, AJ628749
Lysinibacillus irui IRB4-01T, OQ566940
96
Lysinibacillus pakistanensis NCCP-54, AB558495
Lysinibacillus capsici PB300T, PXXX01000000
Lysinibacillus xylanilyticus XDB9T, FJ477040
80
RBPW-LTUS3-MHCIP4-4, PV243509
76
Lysinibacillus sphaericus DSM 28T, AJ310084
75
PLBPW-PTST1-R2ACIP1-2A, PV243480
75
RBPW-LUST3-R2ACIP4-4, PV243525
Lysinibacillus fusiformis DSM 2898T, AJ310083
Lysinibacillus cavernae SYSU K30005T, MN611148
"Paenibacillus taohushanense" gs65T, NR_118393
RBPW-PTST3-R2ACIP1-4, PV243546
Paenibacillus odorifer TOD45T, AJ223990
PLBPW-PTST1-R2ACIP1-1, PV243479
83
Paenibacillus typhae xj7T, JN256679
Paenibacillus tianjinensis TB2019T, MW579055
Paenibacillus auburnensis JJ-7, OP341377
Paenibacillus azotifigens NF2-4-5, KX510118
85
PLBPW-VUST3-MHCIP1-1B, PV243456
RBPW-LUUS2-R2ACIP1-3, PV243524
PLBPW-VUUS1-R2ACIP1-2, PV24346
PLBPW-LTUS2-R2ACIP4-2, PV243496
PLBPW-VUST1-R2ACIP1-1, PV243466
Paenibacillus etheri SH7, KC625558
Paenibacillus borealis KK19, AJ011322
Paenibacillus rhizoplanae JJ-64, KY114946
PLBPW-LUUS1-R2ACIP1-2, PV243499
Paenibacillus mobilis S8T, KX636168
Paenibacillus faecis CIP 101062T, HM212646
75
Paenibacillus cucumis AP-115T, KU201962
Paenibacillus intestini LAH16T, KU573975
Paenibacillus polysaccharolyticus BL9T, EU912452
77
Paenibacillus silvae DB13031T, KC835077
PLBPW-PUST3-R2ACIP1-1C, PV243488
Paenibacillus tundrae A10bT, EU558284
Paenibacillus xylanexedens B22aT, EU558281
90
PLBPW-PTST1-MHCIP1-3, PV243485
Paenibacillus amylolyticus NRRL NRS-290T, D85396
PLBPW-VUUS2-R2ACIP1-2, PV243470
PLBPW-VUUS2-MHCIP1-1A, PV243458
PLBPW-VUUS2-MHCIP1-2, PV243459
Paenibacillus brasilensis PB172T, AF273740
96
Paenibacillus terrae AM141T, AF391124
Paenibacillus ottowii MS2379T, MH842737
Paenibacillus kribbensis AM49T, AF391123
99
Paenibacillus polymyxa DSM 36T, HG324065
0.10

Figure S4. Phylogenetic placement of cultivated bacterial strains from this study based on partial 16S rRNA gene sequences. The phylogenetic tree was calculated in ARB in the LTP type strain database using the Neighbour joining method. Due to a better overview the calculated tree was split in subtrees. A/B: *Pseudomonadota* (*Gammaproteobacteria*); C: *Pseudomonadota* (*Alphaproteobacteria*); D: *Pseudomonadota* (*Betaproteobacteria*), *Campylobacterota; Bacteroidota*; E: *Actinomycetota*; F: *Bacillota*. Numbers at nodes: bootstrap values of 70% and larger. Scale bars: 0.1 substitutions per nucleotide position. Bold: sequences of strains cultivated from surface washed cilantro leaves (green coloured) and roots (brown colours) in this study or by irrigation water types (blue coloured) as described by Soufi *et al.* (2025).

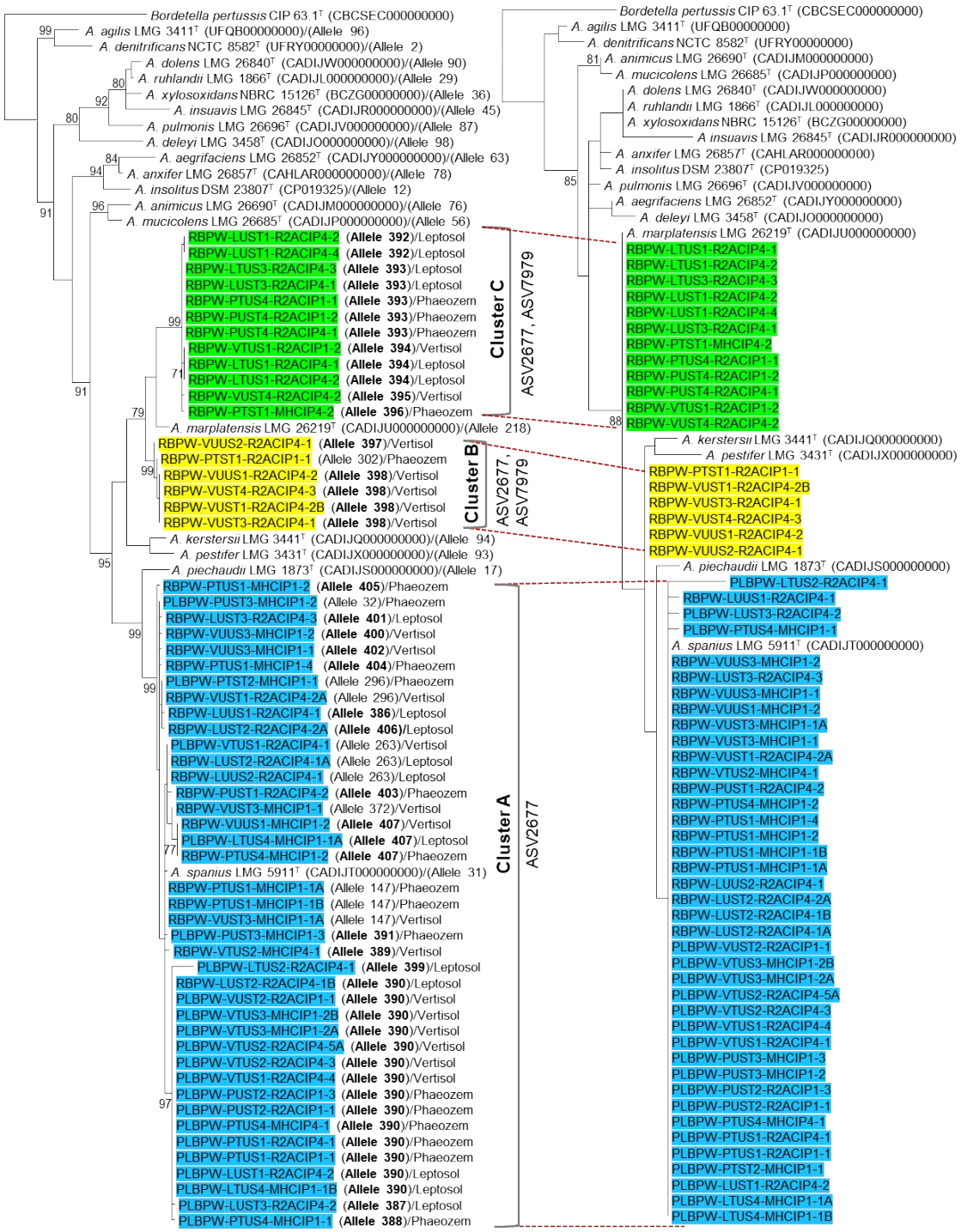

Figure S5. Phylogenetic placement of *Achromobacter* spp. strains within the genus *Achromobacter*. Phylogenetic analyses were performed using Maximum Likelihood method (Felsenstein 1981). Nucleotide based analysis was carried out by the General Time Reversible model (GTR; Nei and Kumar 2000). Amino acid-based analysis was done by the JTT matrix-based model (Jones, Taylor and Thornton. 1992). A discrete Gamma distribution was used to model evolutionary rate differences among sites [5 categories (+G)] assuming some sites to be evolutionarily invariable (+I). The trees of (A) *nrdA* and (B) NrdA were based on total of 765 nucleotides and 255 amino acids positions in the final datasets. All positions containing gaps and missing data were eliminated. Bootstrap values (≥70%) after 100 resamplings are indicated at branch nodes; bar, number of substitutions per site. Evolutionary analyses were conducted in MEGA11 (Tamura *et al.*, 2021). Novel alleles from this study were given in bold font.

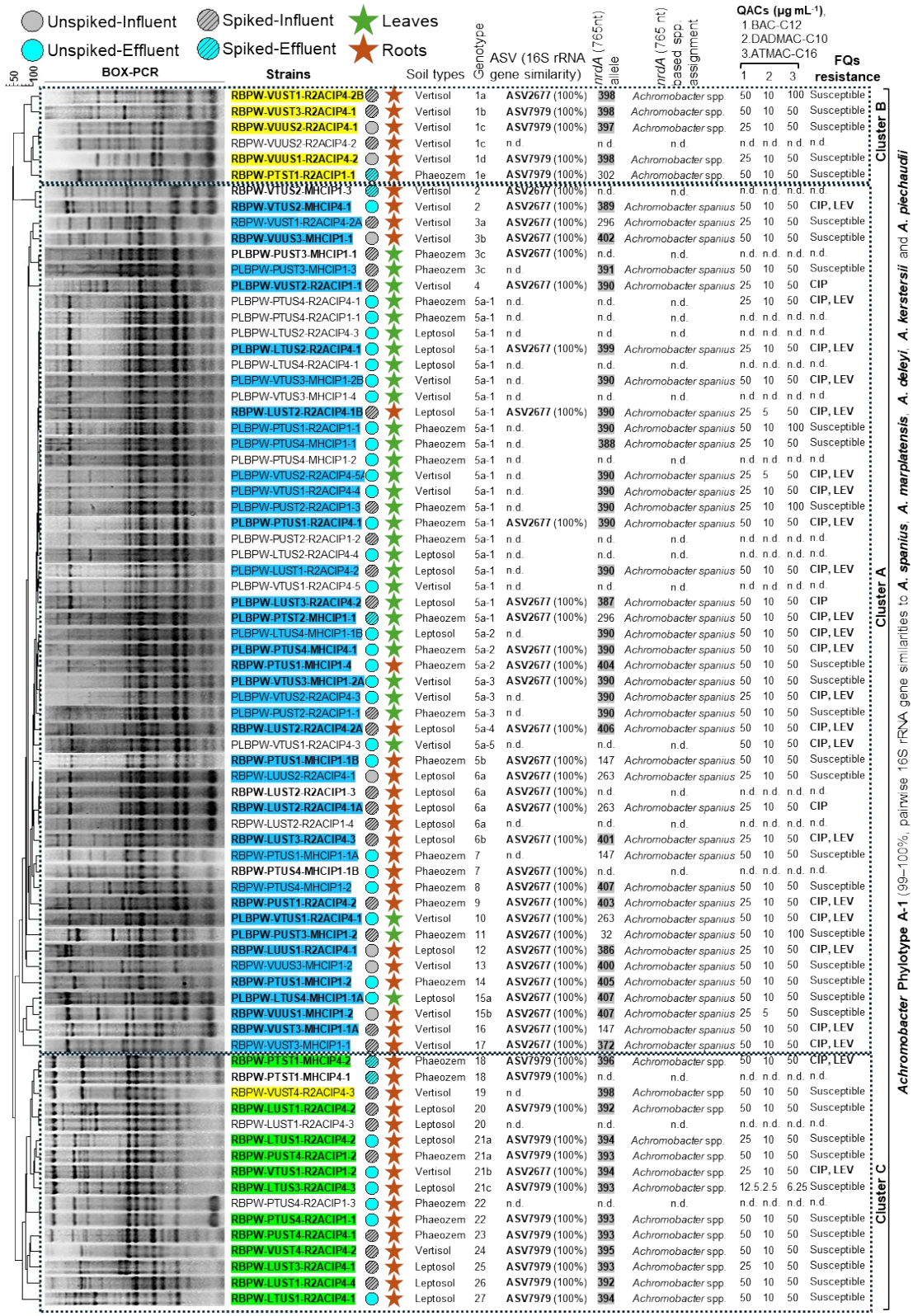

Figure S6. Genotyping of all *Achromobacter* spp. strains based on fingerprint patterns obtained by genomic fingerprinting using primers targeting BOX repetitive elements. Cluster analysis was performed in BioNumerics version 8 (Applied Maths, Belgium) using UPGMA clustering, based on a dissimilarity matrix generated by the Pearson correlation coefficient. Strains in bold font were identified by partial 16S rRNA gene sequencing. Species assignment as *Achromobacter spanius* was based on *nrdA* gene, and species assignment provided inside the brackets were based on identical BOX pattern (same genotype). Allele numbers with bold font represented novel alleles from this study.

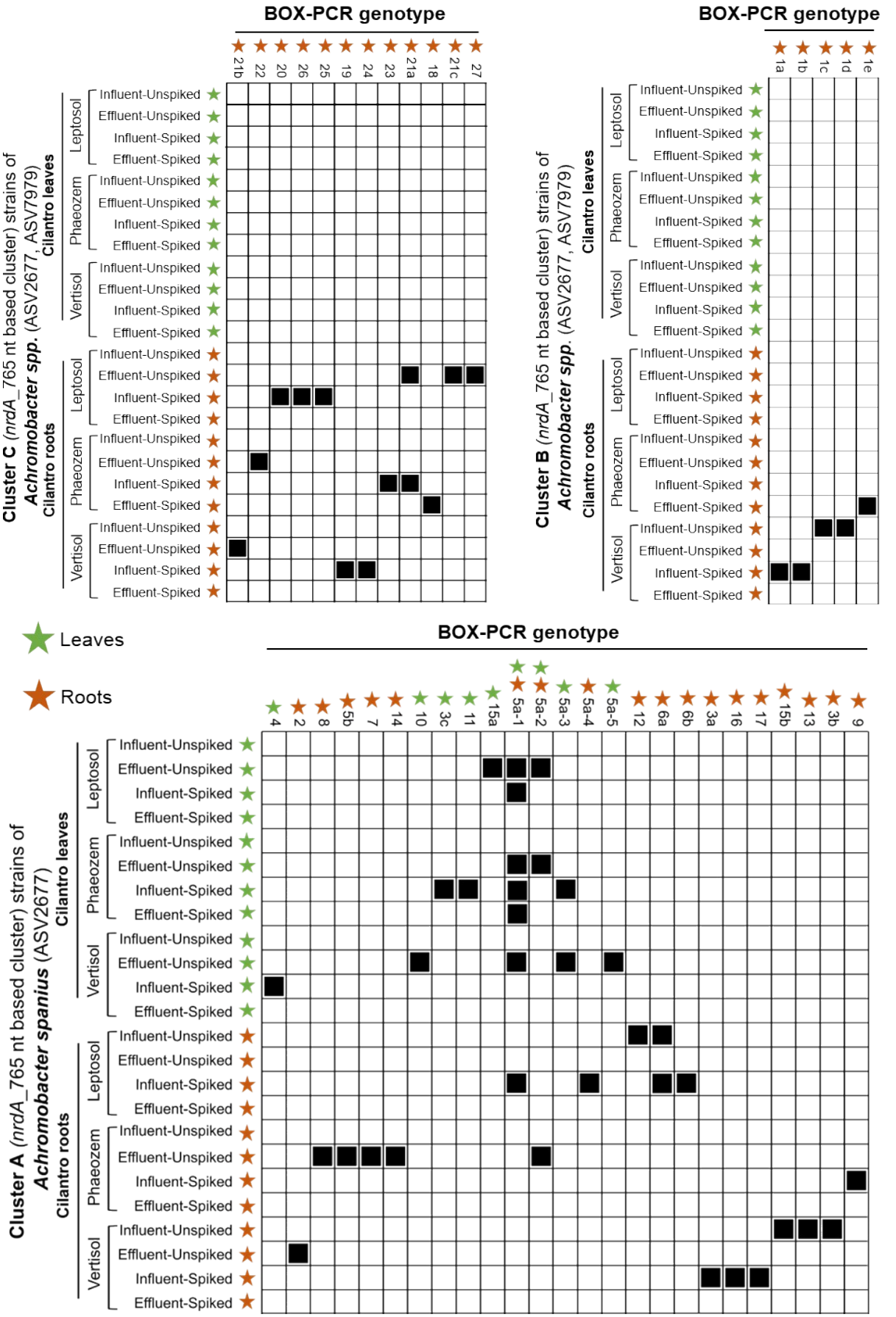

Figure S7. Seriation analysis based on an absence-presence (0/1) matrix illustrating the presence of *Achromobacter* genotypes in the leaves and roots of cilantro grown on different soil types irrigated with different wastewater types.

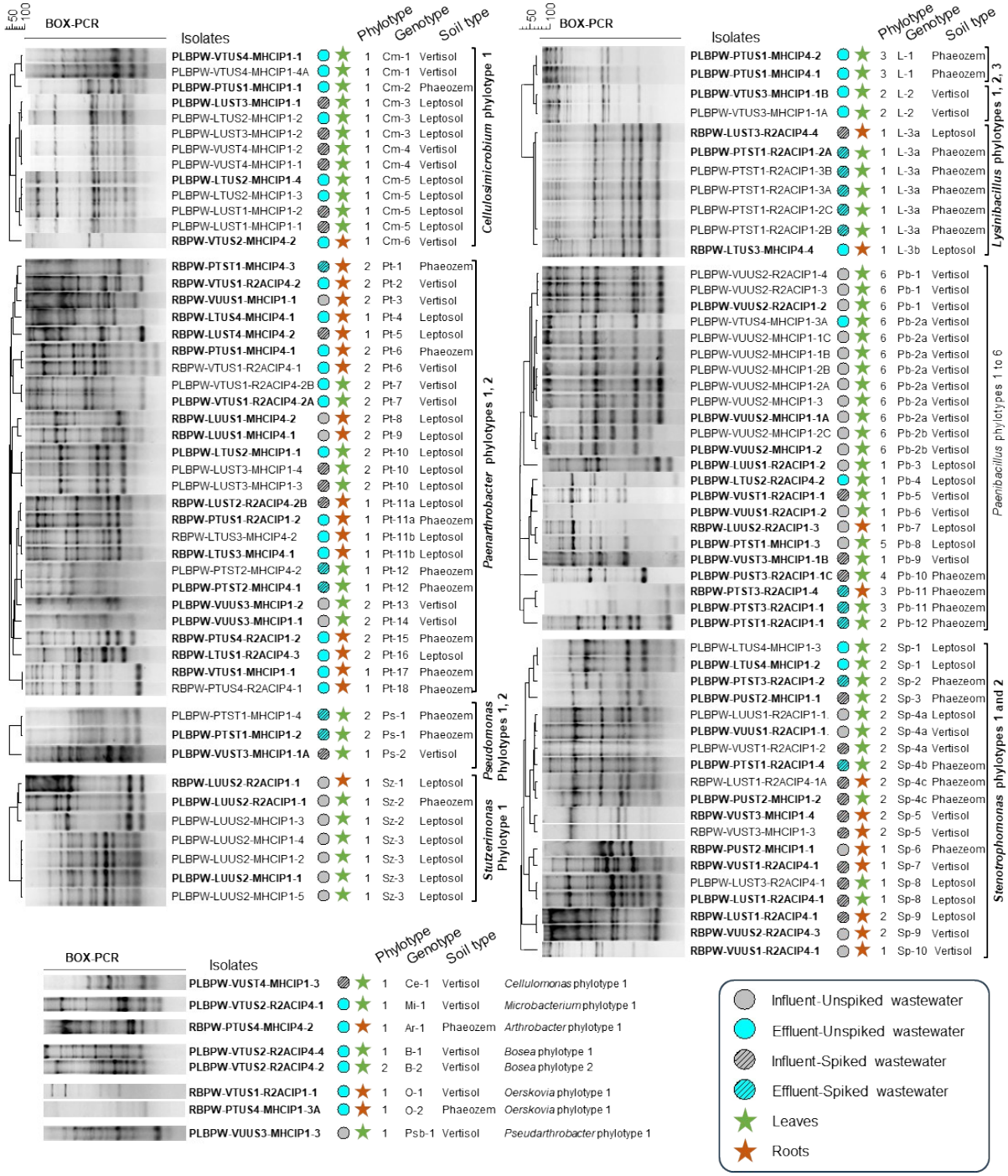

Figure S8. Genotyping of all bacterial phylotypes beside *Achromobacter* based on fingerprint patterns obtained by genomic fingerprinting using primer BOXA1R. Cluster analysis was performed in BioNumerics version 8 (Applied Maths, Belgium) using UPGMA clustering, based on a dissimilarity matrix generated by the Pearson correlation coefficient. Strains in bold font were identified by partial 16S rRNA gene sequencing.

Supplementary reference

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