Supplementary Material

Plant Growth, Antibiotic Uptake, and Prevalence of Antibiotic Resistance in an Endophytic System of Pakchoi under Antibiotic Exposure

Hao Zhang, Xunan Li, Qingxiang Yang, Linlin Sun, Xinxin Yang, Mingming Zhou, Rongzhen Deng, and Linqian Bi

Table S1–S3

Table S1. PCR primers, annealing temperatures, and resistance mechanisms

Table S2. qPCR primers and annealing temperatures used in the present study

Table S3. qPCR standard curves for 16S rRNA gene and antibiotic resistance genes

Table S4. Root length, plant height, and fresh biomass values of hydroponic pakchoi under different dosages of antibiotic treatment

1

Gene	Primer	5'–3' sequence	Resistance Mechanism	Anneal temp. (°C)	Reference
name					
tetA	FW	GCTACATCCTGCTTGCCTTC	efflux pump	55	[1]
	RV	CATAGATCGCCGTGAAGAGG			
tetC	FW	CTTGAGAGCCTTCAACCCAG	efflux pump	55	[1]
	RV	ATGGTCGTCATCTACCTGCC			
tetG	FW	GCTCGGTGGTATCTCTGCTC	efflux pump	55	[1]
	RV	AGCAACAGAATCGGGAACAC			
tetK	FW	CGAAAACAGACTCGCCAATC	efflux pump	55	[1]
	RV	TCCATAATGAGGTGGGGC			
tetL	FW	TCGTTAGCGTGCTGTCATTC	efflux pump	55	[1]
	RV	GTATCCCACCAATGTAGCCG			
tetM	FW	ACAGAAAGCTTATTATATAAC	ribosomal protection protein	45	[2]
	RV	TGGCGTGTCTATGATGTTCAC			
tetO	FW	ACGGARAGTTTATTGTATACC	ribosomal protection protein	45	[2]
	RV	TGGCGTATCTATAATGTTGAC			
tetQ	FW	AGAATCTGCTGTTTGCCAGTG	ribosomal protection protein	55	[2]
	RV	CGGAGTGTCAATGATATTGCA			
tetT	FW	AAGGTTTATTATATAAAAGTG	ribosomal protection protein	45	[2]
	RV	AGGTGTATCTATGATATTTAC			
tetW	FW	GAGAGCCTGCTATATGCCAG	ribosomal protection protein	55	[2]
	RV	GGGCGTATCCACAATGTTAAC			
tetX	FW	CAATAATTGGTGGTGGACCC	enzymatic modification	55	[1]
	RV	TTCTTACCTTGGACATCCCG			
tetB/P	FW	AAAACTTATTATATATATAGTG	ribosomal protection protein	45	[3]

Table S1. PCR primers, annealing temperatures, and resistance mechanisms.

2

	RV	IGGAGIAICAAIAAIAIICAC			
sul1	FW	CGGCGTGGGCTACCTGAACG	ribosomal protection protein	60	[2]
	RV	GCCGATCGCGTGAAGTTCCG			
sul2	FW	GCGCTCAAGGCAGATGGCATT	ribosomal protection protein	60	[4]
	RV	GCGTTTGATACCGGCACCCGT			
sul3	FW	TCAAAGCAAAATGATATGAGC	ribosomal protection protein	50	[5]
	RV	TTTCAAGGCATCTGATAAAGAC			
dfrA1	FW	AGCATTACCCAACCGAAAGT	enzymatic modification	50	[6]
	RV	TGTCAGCAAGATAGCCAGAT			
dfrA7	FW	AAATGGCGTAATCGGTAATG	enzymatic modification	50	[6]
	RV	GTGAACAGTAGACAAATGAAT			
bla _{amp} C	FW	TGGCGTATCGGGTCAATGT	enzymatic modification	55	[5]
	RV	CTCCACGGGCCAGTTGAG			
blavim	FW	GCACTTCTCGCGGAGATTG	enzymatic modification	55	[5]
	RV	CGACGGTGATGCGTACGTT			
blaстх-м	FW	ATGTGCAGYACCAGTAARGTKATGGC	enzymatic modification	55	[7]
	RV	ATCACKCGGRTCGCCNGGRAT			
blatem	FW	TCGGGGAAATGTGCG	enzymatic modification	50	[7]
	RV	GGAATAAGGGCGACA			
<i>bla</i> shv	FW	CTTTCCCATGATGAGCACCTTT	enzymatic modification	55	[5]
	RV	TCCTGCTGGCGATAGTGGAT			
blaz	FW	GGAGATAAAGTAACAAATCCAGTTAGATATGA	enzymatic modification	55	[5]
	RV	TGCTTAATTTTCCATTTGCGATAAG			

Gene name	Primer	5'-3' sequence	Product size (bp)	Anneal temp. (°C)	Reference
16S rRNA	338F	CCTACGGGAGGCAGCAG	202	60	[8]
	518R	ATTACCGCGGCTGCTGG			
tetX	FW	AGCCTTACCAATGGGTGTAAA	278	60	[9]
	RV	TTCTTACCTTGGACATCCCG			
sul1	FW	CCGTTGGCCTTCCTGTAAAG	67	60	[10]
	RV	TTGCCGATCGCGTGAAGT			
sul2	FW	CTCCGATGGAGGCCGGTAT	190	60	[10]
	RV	GGGAATGCCATCTGCCTTGA			

Table S2. qPCR primers and annealing temperatures used in the present study.

GGAGGCGTGACGGCTTTT

TTCAGTGCGATCCAGACGAA

FW

RV

blaстх-м

Table S3. qPCR standard curves for 16S rRNA gene and antibiotic resistance genes.

101

51

[5]

Gene name	Standard curves	R ²
16S rRNA	$Y = -3.407 \times Log(X) + 36.004$	0.99497
tetX	$Y = -3.0462 \times Log(X) + 34.639$	0.99532
sul1	$Y = -2.9326 \times Log(X) + 34.148$	0.99082
sul2	$Y = -3.425 \times Log(X) + 36.19$	0.99686
<i>bla</i> стх-м	$Y = -2.7213 \times Log(X) + 33.879$	0.99391

Antibiotic	Root	ot length		height	Fresh biomass	
concentration (mg L ⁻¹)	average value	inhibition rate	average value	inhibition rate	average value (g)	inhibition rate
TC treatment	(eni)	(70)	(cm)	(70)		(70)
0	$19.8 \pm 3.12 \text{ b}^*$		12.45 ± 1.14 b		10.72 ± 2.46 b	
50%MIC	22.8 ± 2.44 a	-15.15	13.85 ± 1.06 a	-11.24	20.75 ± 3.09 a	-93.53
MIC	15.6 ± 1.51 c	21.21	10.8 ± 1.23 c	13.25	8.01 ± 1.99 c	25.31
CPL treatments						
0	19.8 ± 3.12 b		12.45 ± 1.14 bc		10.72 ± 2.46 b	
50%MIC	25.6 ± 2.79 a	-29.29	14. 5 ± 2.07 a	-16.47	15.06 ± 2.90 a	-40.44
MIC	24.5 ± 3.24 a	-23.73	13.1 ± 1.29 b	-5.22	12.97 ± 1.87 a	-20.99
SMX treatments						
0	19.8 ± 3.12 a		12.45 ± 1.14 b		10.72 ± 2.46 b	
50%MIC	20.3 ± 0.95 a	-2.53	13.95 ± 1.17 a	-12.05	12.73 ± 1.47 a	-18.72
MIC	12.05 ± 2.52 b	39.14	11.15 ± 1.20 c	10.44	6.51 ± 1.53 c	39.28

Table S4. Root length, plant height, and fresh biomass values of hydroponic pakchoi under different dosages of antibiotic treatment.

The values are mean \pm SD (n = 10). * Different letters indicate the differences in the value among the treatments based on the LSD (Least Significant Difference) test (P < 0.05)

5

Commented [Edit1]: Consider defining.

References

- Ng, L.K.; Martin, I.; Alfa, M.; Mulvey, M. Multiplex PCR for the detection of tetracycline resistant genes. *Mol. Cell Probes* 2001, 15, 209–215.
- Aminov, R.I.; Garrigues-Jeanjean, N.; Mackie, R.I. Molecular ecology of tetracycline resistance: development and validation of primers for detection of tetracycline resistance genes encoding ribosomal protection proteins. *Appl. Environ. Microbiol.* 2001, 67, 22–32.
- Mu, Q.; Li, J.; Sun, Y.; Mao, D.; Wang, Q.; Yi, L. Occurrence of sulfonamide-, tetracycline-, plasmidmediated quinolone- and macrolide-resistance genes in livestock feedlots in northern China. *Environ. Sci. Pollut. Res.* 2015, 22, 6932–6940.
- Kerrn, M.B.; Klemmensen, T.; Frimodt-Møller, N.; Espersen, F. Susceptibility of danish *Escherichia coli* strains isolated from urinary tract infections and bacteraemia, and distribution of *sul* genes conferring sulphonamide resistance. *J. Antimicrob. Chemother.* 2002, *50*, 513–516.
- Zhu, Y.G.; Johnson, T.A.; Su, J.Q.; Qiao, M.; Guo, G.X.; Stedtfeld, R.D.; Hashsham, S.A.; Tiedje, J.M. Diverse and abundant antibiotic resistance genes in Chinese swine farms. *Proc. Natl. Acad. Sci. USA* 2013, *110*, 3435–3440.
- Selvam, A.; Xu, D.L.; Zhao, Z.Y.; Wong, J.W.C. Fate of tetracycline, sulfonamide and fluoroquinolone resistance genes and the changes in bacterial diversity during composting of swine manure. *Bioresour. Technol.* 2012, 126, 383–390.
- Knapp, C.W.; Zhang, W.; Sturm, B.S.M.; Graham, D.W. Differential fate of erythromycin and betalactam resistance genes from swine lagoon waste under different aquatic conditions. *Environ. Pollut.* 2010, *158*, 1506–1512.
- Muyzer, G.; De Waal, E.C.; Uitterlinden, A.G. Profiling of complex microbial populations by denaturing gradient gel electrophoresis analysis of polymerase chain reaction-amplified genes coding for 16S rRNA. *Appl. Environ. Microbiol.* **1993**, *59*, 695-700.
- Ghosh, S.; Ramsden, S.J.; LaPara, T.M. The role of anaerobic digestion in controlling the release of tetracycline resistance genes and class 1 integrons from municipal wastewater treatment plants. *Appl. Microbiol. Biot.* 2009, 84, 791–796.
- Heuer, H.; Smalla, K. Manure and sulfadiazine synergistically increased bacterial antibiotic resistance in soil over at least two months. *Environ. Microbiol.* 2007, *9*, 657–666.

6