

Supplementary Information

Manuscript title:

Effects of thermophilic composting on oxytetracycline, sulfamethazine, and corresponding resistance genes in swine manure

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Table S1 PCR primers for target ARGs, integrase genes, and 16S rDNA gene

Category	Target ARG	Primer	Sequence (5'-3')	Reference
EFP TRGs	<i>tetA</i>	<i>tetA</i> -F	GCTACATCCTGCTTGCCTTC	1
		<i>tetA</i> -R	CATAGATCGCCGTGAAGAGG	
	<i>tetC</i>	<i>tetC</i> -F	CTTGAGAGCCTTCAACCCAG	1
		<i>tetC</i> -R	ATGGTCGTCATCTACCTGCC	
	<i>tetG</i>	<i>tetG</i> -F	GCTCGGTGGTATCTCTGCTC	1
		<i>tetG</i> -R	AGCAACAGAATCGGGAACAC	
	<i>tetL</i>	<i>tetL</i> -F	TCGTTAGCGTGCTGTCATTC	1
		<i>tetL</i> -R	GTATCCCACCAATGTAGCCG	
EI TRGs	<i>tetX</i>	<i>tetX</i> -F	CAATAATTGGTGGTGGACCC	1
		<i>tetX</i> -R	TTCTTACCTTGGACATCCCG	
RPP TRGs	<i>tetM</i>	<i>tetM</i> -F	ACAGAAAGCTTATTATATAAC	2
		<i>tetM</i> -R	TGGCGTGTCTATGATGTTTAC	
	<i>tetO</i>	<i>tetO</i> -F	ACGGARAGTTTATTGTATACC	2
		<i>tetO</i> -R	TGGCGTATCTATAATGTTGAC	
	<i>tetQ</i>	<i>tetQ</i> -F	AGAATCTGCTGTTTGCCAGTG	2
		<i>tetQ</i> -R	CGGAGTGTCAATGATATTGCA	
	<i>tetW</i>	<i>tetW</i> -F	GAGAGCCTGCTATATGCCAGC	2
		<i>tetW</i> -R	GGGCGTATCCACAATGTTAAC	
SRGs	<i>sul1</i>	<i>Sul1q</i> -F	CACCGGAAACATCGCTGCA	3
		<i>Sul1q</i> -R	AAGTTCCGCCGCAAGGCT	
	<i>sul2</i>	<i>Sul2q</i> -F	CTCCGATGGAGGCCGGTAT	3
		<i>Sul2q</i> -R	GGGAATGCCATCTGCCTTGA	
Integrans	<i>int11</i>	C1q-786F	GGGTCAAGGATCTGGATTTTCG	4
		C1q-303R	ACATGCGTGTAATCATCGTCG	
	<i>int12</i>	C2q-F	GTTATTTTATTGCTGGGATTAGGC	3
		C2q-R	TTTTACGCTGCTGTATGGTGC	
	16S rDNA ^a	16Sq-F	CCTACGGGAGGCAGCAG	5
		16Sq-R	TTACCGCGGCTGCTGGCAC	
16S rDNA ^b	F984-GC	CGCCCGGGGCGCGCCCCGGGCG GGGCGGGGGCACGGGGGG- AACCGAAGAACCTTAC	6	
	R1378	CGGTGTGTACAAGGCCCGGGAACG		

^a Primer pair used for q-PCR. ^b Primer pair used for PCR-DGGE.

Table S2 q-PCR conditions

Target gene	Annealing	Extending and		Amplification	Limit of detection
	(s)	detection (s)			
	55°C	60°C	72°C	efficiency (%)	(gene copy number)
<i>tetC</i>	20	– ^a	45	105.6	1000
<i>tetG</i>	20	–	45	100.4	10
<i>tetO</i>	20	–	45	99.7	1000
<i>sul2</i>	20	–	45	102.2	100
<i>intI1</i>	20	–	45	103.8	100
<i>intI2</i>	20	–	45	99.4	30
<i>tetA</i>	–	45	–	109.7	100
<i>tetQ</i>	–	45	–	105.3	100
<i>tetW</i>	–	45	–	104.3	10
<i>tetX</i>	–	45	–	101.6	350
16S rDNA	–	45	–	104.4	5000
<i>sul1</i>	–	45	–	105.9	100
<i>tetL</i>	10	–	31	105.3	50
<i>tetM</i>	10	–	31	109.9	10

^a This step was not adopted.

Table S3 Relative abundances of target ARGs and integrase genes in the control and AS (gene copies per 16S rDNA gene)

Day	<i>tetM</i>	<i>tetO</i>	<i>tetQ</i>	<i>tetW</i>	<i>tetA</i>	<i>tetC</i>	<i>tetG</i>	<i>tetL</i>	<i>tetX</i>	<i>intI1</i>	<i>intI2</i>	<i>sul1</i>	<i>sul2</i>
0 ^a	4.32E-03 ^b	6.78E-02	2.14E-03	7.35E-03	1.33E-03	8.44E-05	7.89E-05	9.28E-03	1.40E-03	7.70E-04	8.08E-05	8.20E-03	3.57E-04
	(4.12E-04) ^b	(2.17E-03)	(4.27E-04)	(5.82E-05)	(4.10E-05)	(4.56E-06)	(5.29E-06)	(1.70E-04)	(1.88E-05)	(2.90E-05)	(5.42E-06)	(1.06E-03)	(7.90E-05)
Control													
1	7.17E-03	4.67E-02	1.09E-03	4.74E-03	2.82E-02	3.58E-03	9.54E-03	6.34E-03	7.43E-04	2.36E-02	3.41E-03	2.48E-02	2.68E-02
	(3.94E-03)	(2.86E-02)	(1.61E-04)	(7.13E-04)	(1.81E-02)	(1.94E-03)	(8.95E-03)	(2.70E-03)	(2.81E-04)	(1.36E-02)	(3.14E-03)	(8.38E-04)	(2.12E-02)
3	5.34E-03	7.53E-02	4.52E-04	2.56E-03	5.60E-03	2.32E-03	5.92E-03	7.04E-03	2.75E-04	9.15E-03	5.24E-03	1.04E-02	8.49E-03
	(5.34E-04)	(6.40E-04)	(1.60E-04)	(3.53E-04)	(1.72E-03)	(8.81E-04)	(3.01E-05)	(4.39E-05)	(9.59E-05)	(1.44E-03)	(9.93E-04)	(1.46E-03)	(3.79E-05)
7	5.91E-03	1.36E-02	1.90E-02	8.75E-04	8.56E-03	9.12E-03	2.10E-02	3.85E-03	2.01E-03	2.41E-03	9.19E-04	4.07E-03	2.23E-03
	(3.55E-03)	(1.13E-03)	(7.21E-03)	(5.63E-04)	(3.29E-04)	(1.20E-02)	(2.70E-02)	(3.75E-04)	(2.13E-03)	(1.07E-03)	(7.58E-04)	(1.37E-04)	(3.89E-04)
12	2.38E-03	5.21E-02	9.25E-07	5.28E-04	2.47E-03	2.50E-04	3.06E-02	5.62E-03	1.68E-02	8.50E-03	2.64E-03	3.08E-02	4.58E-02
	(3.83E-04)	(4.00E-04)	(2.14E-07)	(7.69E-05)	(2.23E-04)	(1.94E-04)	(2.90E-03)	(5.57E-04)	(3.90E-03)	(7.52E-04)	(2.28E-03)	(6.71E-05)	(2.43E-03)
18	1.13E-03	2.71E-02	1.28E-06	2.66E-04	1.40E-03	4.94E-04	1.07E-01	5.87E-03	6.58E-02	2.09E-02	2.19E-02	7.20E-02	1.55E-01
	(3.57E-04)	(6.41E-03)	(1.79E-07)	(6.69E-05)	(1.54E-05)	(2.41E-04)	(5.51E-03)	(1.43E-03)	(4.29E-03)	(1.44E-03)	(2.00E-03)	(3.26E-03)	(1.51E-02)
25	9.48E-04	5.53E-02	1.34E-06	2.10E-04	1.21E-03	9.28E-04	1.49E-01	4.88E-03	4.78E-02	2.02E-02	1.40E-02	1.52E-01	2.04E-01
	(5.03E-05)	(3.68E-03)	(8.11E-07)	(2.18E-05)	(2.91E-04)	(5.27E-04)	(1.79E-02)	(2.48E-04)	(1.41E-02)	(3.80E-04)	(3.66E-03)	(5.07E-03)	(1.77E-02)
32	1.33E-03	4.56E-02	7.60E-07	1.64E-04	1.11E-03	3.90E-04	1.50E-01	4.38E-03	2.59E-02	1.78E-02	6.18E-03	1.35E-01	2.02E-01
	(5.88E-05)	(2.55E-03)	(2.38E-07)	(7.41E-06)	(6.23E-05)	(3.08E-05)	(1.47E-02)	(6.64E-04)	(4.04E-03)	(3.42E-03)	(2.41E-03)	(1.62E-02)	(8.42E-02)
AS													
1	8.59E-03	6.11E-02	1.19E-02	8.61E-03	2.54E-02	1.95E-04	4.59E-04	6.60E-03	8.44E-04	7.35E-03	4.77E-04	7.29E-03	4.85E-03
	(7.66E-03)	(5.09E-02)	(1.28E-02)	(7.24E-04)	(2.72E-02)	(1.03E-04)	(2.92E-04)	(3.23E-03)	(5.18E-05)	(3.90E-03)	(3.27E-04)	(2.26E-03)	(3.22E-03)
3	1.01E-02	1.12E-01	6.83E-04	5.49E-03	1.80E-02	5.14E-04	2.47E-02	1.52E-02	9.11E-04	1.30E-02	6.61E-03	2.48E-02	5.85E-03
	(2.46E-03)	(1.81E-02)	(9.63E-05)	(4.01E-04)	(1.91E-04)	(2.01E-04)	(1.18E-03)	(1.34E-03)	(3.91E-05)	(1.16E-03)	(5.01E-04)	(2.75E-03)	(3.65E-04)
7	3.67E-03	1.02E-01	8.39E-06	2.43E-03	1.58E-02	1.07E-03	6.60E-02	2.40E-02	5.55E-03	2.71E-02	2.58E-04	5.14E-02	6.60E-03

	(1.71E-04)	(7.26E-03)	(6.33E-07)	(2.89E-04)	(3.62E-03)	(2.28E-04)	(3.03E-03)	(3.27E-03)	(1.11E-03)	(1.57E-03)	(2.22E-05)	(4.63E-03)	(6.66E-04)
12	3.78E-03	1.03E-01	2.14E-06	1.67E-03	3.29E-02	5.01E-03	1.74E-01	1.18E-02	8.44E-02	5.80E-02	3.77E-03	1.37E-01	1.21E-01
	(8.05E-04)	(2.88E-02)	(6.34E-07)	(1.74E-04)	(1.46E-03)	(3.44E-03)	(6.50E-02)	(4.03E-03)	(2.74E-03)	(1.45E-02)	(3.06E-03)	(7.16E-03)	(3.91E-02)
18	2.07E-03	5.10E-02	1.07E-06	4.76E-04	4.83E-02	6.29E-03	3.54E-01	1.50E-02	3.81E-01	8.06E-02	2.27E-02	2.40E-01	4.13E-01
	(4.78E-04)	(1.33E-02)	(1.86E-07)	(8.69E-05)	(7.08E-03)	(1.24E-03)	(2.83E-03)	(1.53E-03)	(2.73E-04)	(2.44E-02)	(7.13E-04)	(5.39E-02)	(6.07E-02)
25	9.01E-04	6.16E-02	1.86E-06	3.30E-04	4.10E-02	4.06E-03	4.03E-01	1.07E-02	1.99E-01	5.76E-02	1.59E-02	2.77E-01	3.53E-01
	(1.01E-04)	(2.88E-03)	(5.60E-07)	(1.70E-05)	(1.68E-03)	(9.45E-04)	(6.96E-02)	(1.70E-04)	(2.35E-02)	(1.21E-02)	(2.54E-03)	(1.04E-01)	(9.58E-03)
32	9.36E-04	4.88E-02	7.05E-07	2.32E-04	3.17E-02	4.92E-03	3.01E-01	8.43E-03	1.61E-01	4.34E-02	1.34E-02	2.07E-01	2.82E-01
	(8.56E-04)	(3.35E-02)	(6.70E-07)	(1.00E-04)	(1.47E-02)	(2.63E-03)	(2.60E-01)	(3.93E-03)	(8.62E-02)	(2.39E-02)	(1.37E-02)	(2.07E-02)	(1.66E-01)

^a The data on Day 0 represent the initial relative abundances of target ARGs and integrase genes for both the control and AS. ^b Mean (standard deviation), $n = 3$.

Table S4 Absolute abundances of target ARGs, integrase genes, and 16S rDNA in the control and AS (gene copies per gram, fw)

Day	<i>tetM</i>	<i>tetO</i>	<i>tetQ</i>	<i>tetW</i>	<i>tetA</i>	<i>tetC</i>	<i>tetG</i>	<i>tetL</i>	<i>tetX</i>	<i>intI1</i>	<i>intI2</i>	<i>sul1</i>	<i>sul2</i>	16S rDNA
0 ^a	2.19E+09 ^b	3.40E+10	1.04E+09	3.67E+09	6.61E+08	4.19E+07	3.97E+07	4.63E+09	7.01E+08	3.87E+08	4.01E+07	4.16E+09	1.74E+08	5.00E+11
	(7.19E+08) ^b	(9.13E+09)	(4.02E+07)	(9.01E+08)	(1.37E+08)	(7.73E+06)	(1.20E+07)	(1.02E+09)	(1.57E+08)	(1.06E+08)	(6.88E+06)	(1.50E+09)	(2.82E+06)	(1.19E+11)
Control														
1	6.79E+09	4.41E+10	1.07E+09	4.57E+09	2.65E+10	3.39E+09	8.86E+09	6.04E+09	7.09E+08	2.24E+10	3.17E+09	2.41E+10	2.51E+10	9.71E+11
	(3.19E+09)	(2.37E+10)	(2.52E+08)	(2.75E+08)	(1.51E+10)	(1.56E+09)	(7.84E+09)	(2.06E+09)	(2.07E+08)	(1.11E+10)	(2.75E+09)	(1.37E+09)	(1.82E+10)	(8.80E+10)
3	2.06E+09	2.93E+10	1.81E+08	1.01E+09	2.23E+09	9.31E+08	2.31E+09	2.74E+09	1.10E+08	3.52E+09	2.07E+09	4.08E+09	3.30E+09	5.84E+11
	(1.25E+08)	(4.44E+09)	(9.02E+07)	(2.96E+08)	(1.02E+09)	(4.87E+08)	(3.80E+08)	(4.55E+08)	(5.44E+07)	(8.90E+06)	(7.13E+08)	(1.21E+09)	(5.43E+08)	(9.33E+10)
7	4.81E+09	1.12E+10	1.58E+10	7.11E+08	7.05E+09	7.31E+09	1.69E+10	3.17E+09	1.62E+09	1.97E+09	7.69E+08	3.35E+09	1.83E+09	1.24E+12
	(2.73E+09)	(4.85E+08)	(6.56E+09)	(4.35E+08)	(9.97E+06)	(9.58E+09)	(2.16E+10)	(1.82E+08)	(1.69E+09)	(8.06E+08)	(6.55E+08)	(2.04E+07)	(2.48E+08)	(4.91E+10)
12	5.55E+08	1.22E+10	2.15E+05	1.23E+08	5.76E+08	5.68E+07	7.19E+09	1.31E+09	3.90E+09	2.00E+09	6.38E+08	7.21E+09	1.07E+10	3.51E+11
	(5.01E+07)	(7.73E+08)	(3.47E+04)	(9.23E+06)	(1.10E+07)	(4.12E+07)	(1.19E+09)	(3.68E+07)	(6.33E+08)	(3.17E+08)	(5.78E+08)	(4.97E+08)	(1.93E+08)	(2.50E+10)
18	1.14E+08	2.74E+09	1.29E+05	2.69E+07	1.42E+08	4.95E+07	1.08E+10	5.95E+08	6.64E+09	2.11E+09	2.22E+09	7.26E+09	1.57E+10	1.51E+11
	(3.96E+07)	(7.33E+08)	(1.40E+04)	(7.59E+06)	(6.02E+06)	(2.27E+07)	(2.15E+08)	(1.63E+08)	(2.24E+08)	(7.90E+07)	(2.72E+08)	(9.99E+07)	(2.02E+09)	(4.77E+09)
25	2.15E+08	1.26E+10	2.99E+05	4.78E+07	2.77E+08	2.08E+08	3.39E+10	1.11E+09	1.09E+10	4.58E+09	3.19E+09	3.45E+10	4.64E+10	2.27E+11
	(2.08E+06)	(1.38E+09)	(1.71E+05)	(7.01E+06)	(7.81E+07)	(1.11E+08)	(5.52E+09)	(1.04E+08)	(3.66E+09)	(1.13E+08)	(9.68E+08)	(2.65E+09)	(6.03E+09)	(9.85E+09)
32	2.92E+08	1.01E+10	1.62E+05	3.63E+07	2.44E+08	8.51E+07	3.26E+10	9.49E+08	5.60E+09	3.84E+09	1.41E+09	2.94E+10	4.26E+10	2.20E+11
	(4.56E+07)	(2.56E+09)	(1.90E+04)	(8.83E+06)	(3.52E+07)	(1.03E+07)	(3.34E+09)	(4.60E+07)	(2.45E+08)	(2.84E+07)	(8.01E+08)	(2.39E+09)	(9.65E+09)	(4.39E+10)
AS														
1	5.64E+09	4.05E+10	7.62E+09	6.29E+09	2.13E+10	1.35E+08	3.12E+08	4.57E+09	6.28E+08	5.07E+09	3.23E+08	5.18E+09	3.28E+09	7.38E+11
	(4.08E+09)	(2.64E+10)	(7.27E+09)	(1.04E+09)	(2.48E+10)	(4.06E+07)	(1.32E+08)	(1.18E+09)	(1.92E+08)	(1.54E+09)	(1.54E+08)	(3.37E+08)	(1.49E+09)	(1.83E+11)
3	4.34E+09	4.75E+10	2.90E+08	2.31E+09	7.49E+09	2.24E+08	1.02E+10	6.29E+09	3.78E+08	5.36E+09	2.78E+09	1.05E+10	2.46E+09	4.17E+11
	(1.98E+09)	(1.81E+10)	(1.04E+08)	(6.83E+08)	(1.61E+09)	(1.32E+08)	(1.82E+09)	(8.73E+08)	(6.92E+07)	(7.37E+08)	(8.30E+08)	(3.47E+09)	(7.02E+08)	(9.40E+10)
7	1.44E+09	3.99E+10	3.25E+06	9.56E+08	6.05E+09	4.10E+08	2.57E+10	9.26E+09	2.14E+09	1.05E+10	9.99E+07	1.99E+10	2.56E+09	3.90E+11

	(2.64E+08)	(8.29E+09)	(2.03E+05)	(2.43E+08)	(5.68E+08)	(3.18E+07)	(2.36E+09)	(1.14E+07)	(1.36E+08)	(8.43E+08)	(5.16E+06)	(9.53E+08)	(9.43E+07)	(5.36E+10)
12	1.15E+09	3.15E+10	6.54E+05	5.05E+08	9.95E+09	1.56E+09	5.35E+10	3.62E+09	2.55E+10	1.77E+10	1.18E+09	4.14E+10	3.72E+10	3.01E+11
	(3.59E+08)	(1.18E+10)	(2.57E+05)	(1.04E+08)	(1.45E+09)	(1.19E+09)	(2.49E+10)	(1.58E+09)	(3.42E+09)	(6.16E+09)	(1.04E+09)	(6.37E+09)	(1.55E+10)	(3.07E+10)
18	3.43E+08	8.46E+09	1.78E+05	7.91E+07	8.03E+09	1.05E+09	5.90E+10	2.50E+09	6.35E+10	1.34E+10	3.79E+09	3.97E+10	6.86E+10	1.67E+11
	(6.44E+07)	(1.83E+09)	(2.31E+04)	(1.10E+07)	(8.26E+08)	(2.53E+08)	(3.07E+09)	(1.45E+08)	(2.75E+09)	(3.48E+09)	(4.84E+07)	(7.22E+09)	(7.09E+09)	(7.35E+09)
25	1.59E+08	1.09E+10	3.27E+05	5.83E+07	7.25E+09	7.19E+08	7.15E+10	1.90E+09	3.52E+10	1.01E+10	2.80E+09	4.93E+10	6.24E+10	1.77E+11
	(1.36E+07)	(2.18E+08)	(9.02E+04)	(1.45E+06)	(4.90E+08)	(1.86E+08)	(1.42E+10)	(2.08E+07)	(5.08E+09)	(1.86E+09)	(3.74E+08)	(1.97E+10)	(3.36E+09)	(4.72E+09)
32	1.73E+08	9.03E+09	1.31E+05	4.30E+07	5.86E+09	9.11E+08	5.57E+10	1.56E+09	2.98E+10	8.04E+09	2.48E+09	3.83E+10	5.22E+10	1.85E+11
	(1.59E+08)	(6.22E+09)	(1.24E+05)	(1.87E+07)	(2.74E+09)	(4.89E+08)	(4.83E+10)	(7.32E+08)	(1.61E+10)	(4.45E+09)	(2.54E+09)	(3.96E+09)	(3.08E+10)	(6.62E+08)

^a The data on Day 0 represent the initial absolute abundances of target ARGs and integrase genes for both the control and AS. ^b Mean (standard deviation), $n = 3$.

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