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Supplementary Materials

Tetracycline Induces the Formation of Biofilm of Bacteria from Different Phases of Wastewater Treatment

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Table S1. Concentration (ng/μL) and purity (A260/A280) of DNA samples from the wastewater of the nitrification and sedimentation tanks of the wastewater treatment plant for the quantification of antibiotic resistance genes.

Sample	Wastewater			
	Nitrification tank		Sedimentation tank	
	Concentration (ng/μL)	Purity A260/A280	Concentration (ng/μL)	Purity A260/A280
1	112.000	1.998	30.900	2.002
2	98.000	2.000	14.800	1.833
3	126.000	1.965	17.200	2.053
4	89.000	1.876	21.600	2.100
5	76.000	1.997	22.300	1.984
6	107.000	2.005	34.100	1.937

Table S2. Primers sequences and PCR and qPCR conditions used for detection and quantification of *tet* genes in wastewater samples and selected bacterial isolates.

Primer	Gene	Sequence (5′ to 3′)	PCR and qPCR conditions		
			Annealing temp. (°C)	Cycles	Reference
F1048 R1194	16S rDNA	GTG STG CAY GGY TGT CGT CA ACG TCR TCC MCA CCT TCC TC	60	40	Marti et al. 2013
tet(A)-F tet(A)-R	tetA	CAG CCT CAA TTT CCT GAC GGG CTG GAA GCG AGC GGG TTG AGA G	60	45	Boerjesson et al.2010
tet(B)-F tet(B)-R	tetB	CAG CAA GTG CGC TTT GGA TGC TG TGA GGT GGT ATC GGC AAT GA	60	45	Boerjesson et al.2010
tet(M)-F tet(M)-R	tetM	GCA GAA TAT ACC ATT CAC ATC GAA GT AAA CCA ATG GAA GCC CAG AA	60	40	Rathnayake et al.2012
tet(O)-F tet(O)-R	tetO	ACG GAR AGT TTA TTG TAT ACC TGG CGT ATC TAT AAT GTT GAC	50	40	Marti et al. 2013
tet(W)-F tet(W)-R	tetW	GAG AGC CTG CTA TAT GCC AGC GGG CGT ATC CAC AAT GTT AAC	60	40	Marti et al. 2013

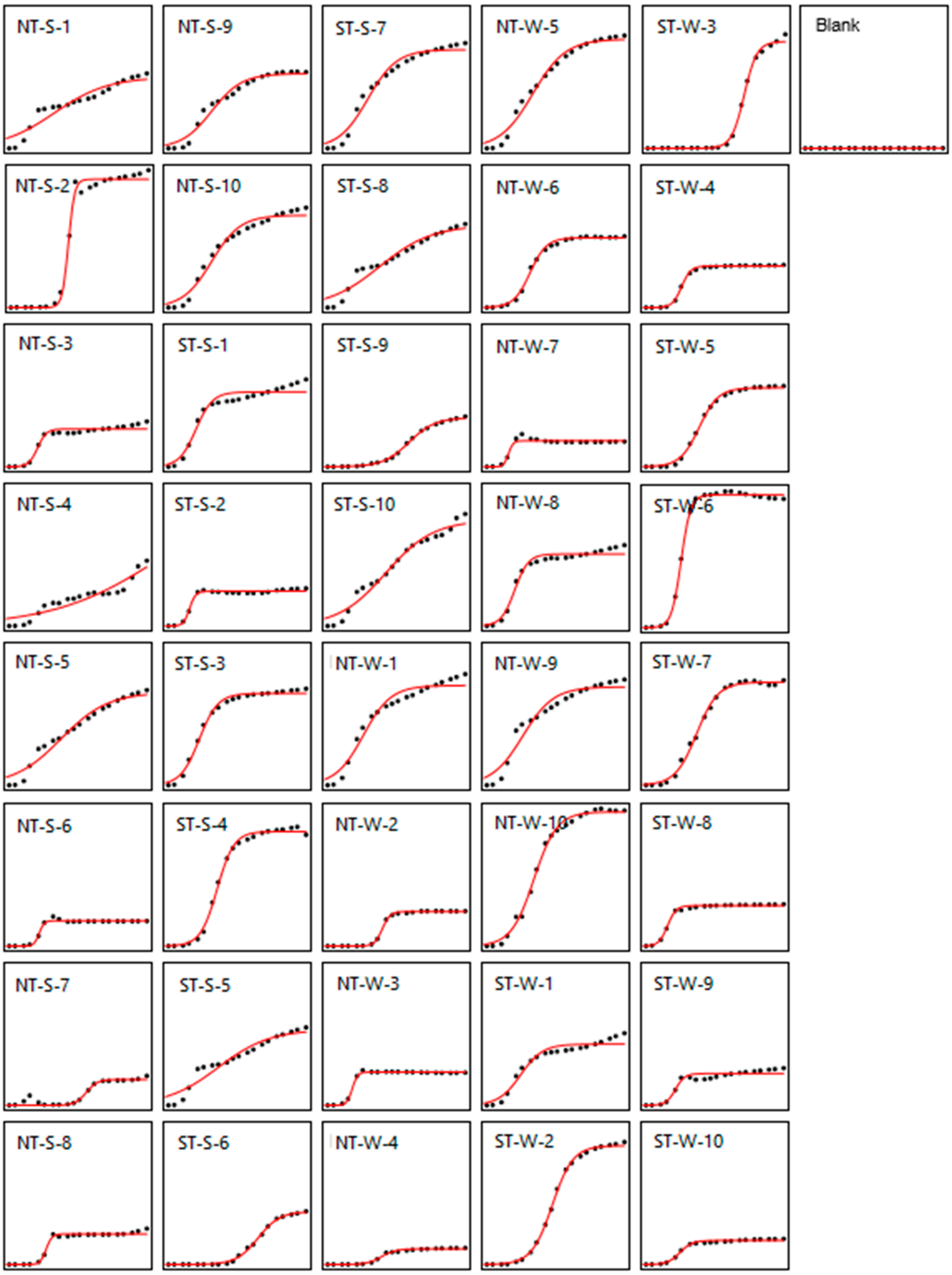

Figure S1. The growth curves of tetracycline-resistant isolates from the nitrification tank (NT) and the sedimentation tank (ST) of the wastewater treatment plant for the preparation of the hydrophobicity test. The growth curves measured every 30 min 24 h at 600 nm and 30 °C.

Table S3. The parameters of the growth curves of tetracycline-resistant isolates from the nitrification tank (NT) and the sedimentation tank (ST) of the wastewater treatment plant for the preparation of the hydrophobicity test. The growth curves measured every 30 min 24 h at 600 nm and 30 °C.

Sample	Growth rate (h)	lag phase (h)	Doubling time (h)
NT-S-1	0.220 ± 0.013	3.120 ± 0.114	3.157 ± 0.181
NT-S-2	1.949 ± 0.063	9.236 ± 0.101	0.356 ± 0.076
NT-S-3	1.356 ± 0.361	3.830 ± 0.293	0.534 ± 0.129
NT-S-4	0.115 ± 0.028	3.476 ± 0.497	6.287 ± 0.534
NT-S-5	0.252 ± 0.001	2.906 ± 0.211	2.752 ± 0.016
NT-S-6	2.424 ± 0.053	4.739 ± 0.153	0.286 ± 0.006
NT-S-7	1.094 ± 0.093	12.269 ± 0.334	0.637 ± 0.057
NT-S-8	2.089 ± 0.142	5.802 ± 0.142	0.333 ± 0.023
NT-S-9	0.392 ± 0.013	0.983 ± 0.750	1.768 ± 0.059
NT-S-10	0.410 ± 0.008	1.177 ± 0.713	1.692 ± 0.035
ST-S-1	0.717 ± 0.068	1.950 ± 0.421	0.972 ± 0.088
ST-S-2	1.998 ± 0.046	3.012 ± 0.132	0.347 ± 0.008
ST-S-3	0.633 ± 0.015	1.907 ± 0.295	1.096 ± 0.026
ST-S-4	0.702 ± 0.013	5.621 ± 0.165	0.987 ± 0.018
ST-S-5	0.228 ± 0.003	3.244 ± 0.124	3.034 ± 0.043
ST-S-6	1.079 ± 0.322	11.532 ± 0.108	0.691 ± 0.247
ST-S-7	0.405 ± 0.006	1.221 ± 0.508	1.711 ± 0.024
ST-S-8	0.225 ± 0.003	3.386 ± 0.206	3.075 ± 0.034
ST-S-9	0.430 ± 0.163	9.512 ± 0.169	1.750 ± 0.544
ST-S-10	0.247 ± 0.004	2.996 ± 0.112	2.806 ± 0.047
NT-W-1	0.425 ± 0.027	0.744 ± 0.804	1.636 ± 0.108
NT-W-2	1.539 ± 0.051	8.402 ± 0.077	0.451 ± 0.015
NT-W-3	2.290 ± 0.025	3.749 ± 0.059	0.303 ± 0.003
NT-W-4	0.824 ± 0.026	6.960 ± 0.268	0.842 ± 0.027
NT-W-5	0.358 ± 0.003	0.807 ± 0.558	1.934 ± 0.017
NT-W-6	0.744 ± 0.128	4.693 ± 0.176	0.950 ± 0.157
NT-W-7	2.618 ± 0.181	3.250 ± 0.179	0.266 ± 0.018
NT-W-8	0.872 ± 0.050	2.756 ± 0.343	0.796 ± 0.048
NT-W-9	0.384 ± 0.011	3.531 ± 0.254	1.808 ± 0.053
NT-W-10	0.517 ± 0.021	3.956 ± 0.277	1.343 ± 0.053
ST-W-1	0.534 ± 0.066	1.520 ± 0.785	1.313 ± 0.172
ST-W-2	0.569 ± 0.005	7.681 ± 0.146	1.218 ± 0.011
ST-W-3	0.922 ± 0.084	12.902 ± 0.415	0.752 ± 0.014
ST-W-4	1.300 ± 0.010	4.807 ± 0.113	0.533 ± 0.004
ST-W-5	0.616 ± 0.003	5.762 ± 0.156	1.125 ± 0.005
ST-W-6	1.245 ± 0.071	4.379 ± 0.061	0.557 ± 0.062
ST-W-7	0.553 ± 0.014	4.881 ± 0.124	1.254 ± 0.032
ST-W-8	1.384 ± 0.078	2.754 ± 0.143	0.502 ± 0.028
ST-W-9	1.288 ± 0.078	4.145 ± 0.355	0.540 ± 0.033
ST-W-10	0.976 ± 0.077	4.041 ± 0.234	0.713 ± 0.057

Table 4. Efficiency of qPCR assays retrieved from standard curves.

qPCR assay	Efficiency (%)	R^2	Limit of quantification (copy number)
rDNA	97.470	0.996	24.280
*tet*W	99.050	0.998	23.400
*tet*B	97.390	0.998	27.710