

Supplementary

Removal of Antibiotic Resistance Genes, Class 1 Integrase Gene and *Escherichia coli* Indicator Gene in a Microalgae-Based Wastewater Treatment System

Table S1 Primer pairs and annealing temperatures used in the current study.

Primer	Primer sequence (5'-3')	Annealing Temp./Number of cycles	Reference
338-F	ACTCCTACGGGAGGCAGCAG	58°C/35	Yan et al. (2018)
518-R	ATTACCGCGGCTGCTGG		
intI1-F	GGCTTCGTATGCCTGCTT	55°C/35	Chen et al. (2016)
intI1-R	CATT CCTGCCGTGGTTCT		
tetO-F	ACGGARAGTTATTGTATAACC	55°C/35	Al-Jassim et al. (2015)
tetO-R	TGGCGTATCTATAATGTTGAC		
tetW-F	GAGAGCCTGCTATATGCCAGC	60°C/40	Al-Jassim et al. (2015)
tetW-R	GGCGTATCCACAATGTTAAC		
tetX-F	AGCCTTACCAATGGGTGTAAC	60°C/40	Dong et al. (2019)
tetX-R	TTCTTACCTTGGACATCCCG		
blaTEM-F	AGCATCTTACGGATGGCATGA	60°C /40	Gao et al. (2020)
blaTEM-R	TCCTCCGATCGTTGTCAGAAGT		
blaCTX-F	AGTGAAAGCGAACCGAACATC	55°C/40	Wen et al. (2016)
blaCTX-R	CTGTCACCAATGCTTTACC		
ermB-F	AAAACTTACCCGCCATACCA	55°C/40	Knapp et al. (2010)
ermB-R	TTTGGCGTGTTCATTGCTT		
aadA-F	GTTGTGCACGACGACATCAT	55°C/35	Su et al. (2020)
aadA-R	TGGTGGTACTTCATCGGCATA		
sul1-F	CACCGGAAACATCGCTGCA	55°C/35	Cheng et al. (2020)
sul1-R	AAGTTCCGCCGCAAGGCT		
sul2-F	GCGCTCAAGGCAGATGGCATT	55°C/40	Cheng et al. (2020)
sul2-R	GCGTTGATACCGGCACCCGT		
cmlA-F	GCCAGCAGTGCCGTTAT	55°C/40	Chen et al. (2016)
cmlA-R	GGCCACCTCCCAGTAGAA		
floR-F	CGGT CGGTATTGTCTTCACG	58°C/40	Chen et al. (2016)
floR-R	TCACGGGCCACGCTGTAT		
uidA-F	AAAACGGCAAGAAAAAGCAG	60°C/40	Adekanmbi et al. (2020)
uidA-R	ACCGTGGTTACAGTCTTGCG		

A CM2

	aadA	CTX	TEM	cmlA	floR	sul1	sul2	int1	uidA	TDS	pH	EC	TSS
aadA	1	0.780	0.809	0.687	0.242	0.822 *	0.929 **	0.97 **	0.85 *	0.871 *	-0.947 * *	0.877 *	-0.873 *
CTX		1	0.998 **	0.986 * *	0.708	0.986 **	0.953 **	0.615	0.991 **	0.481	-0.790	0.485	-0.914
TEM			1	0.98 **	0.683	0.984 **	0.967 **	0.654	0.996 **	0.512	-0.806	0.516	-0.922 *
cmlA				1	0.798	0.954	0.899 *	0.512	0.96 **	0.372	-0.701	0.375	-0.88 *
floR					1	0.670	0.509	0.073	0.623	0.037	-0.352	0.034	-0.625
sul1						1	0.96 **	0.670	0.983 **	0.588	-0.869 *	0.590	-0.949 **
sul2							1	0.814 *	0.984 **	0.674	-0.897 *	0.680	-0.929 **
int1								1	0.704	0.928 **	-0.898 *	0.933 **	-0.782
uidA									1	0.559	-0.836 *	0.564	-0.924 *
NO3										0.716	-0.413	0.721	-0.131
TDS										1	-0.906 *	1 ***	-0.752
pH											1	-0.907 *	0.928 **
Temp.												0.138	0.130
EC												1	-0.752
TSS													1

B KL10

	aadA	CTX	TEM	cmlA	floR	sul1	sul2	int1	uidA	TDS	pH	EC	TSS
aadA	1	0.989 **	0.958 **	0.963 **	0.438	0.790	0.9 *	0.812 *	0.917 **	0.542	-0.714	0.543	-0.869 *
CTX		1	0.988 **	0.929 **	0.303	0.807	0.947 **	0.844 *	0.966 **	0.560	-0.765	0.561	-0.905 *
TEM			1	0.898 *	0.168	0.858 *	0.962 **	0.899 *	0.99 **	0.638	-0.843 *	0.638	-0.95 **
cmlA				1	0.524	0.856 *	0.791	0.849 *	0.826 *	0.673	-0.752	0.674	-0.864 *
floR					1	0.120	0.098	0.040	0.052	-0.020	0.114	-0.016	-0.050
sul1						1	0.762	0.988 **	0.813 *	0.938 **	-0.969	0.939 **	-0.949 **
sul2							1	0.805	0.979 **	0.519	-0.765	0.518	-0.866 *
int1								1	0.869 *	0.905 *	-0.982 **	0.905 *	-0.982 **
uidA									1	0.589	-0.829 *	0.588	-0.93 **
NO3										0.688	-0.437	0.688	-0.175
TDS										1	-0.929 **	1	-0.811 *
pH											1	-0.928 **	0.945 **
Temp.												0.117	0.123
EC												1	-0.811
TSS													1

Figure S1. Correlation of the study genes with physicochemical water quality parameters in the monocultures. ** Correlation is significant at the 0.01 level (2-tailed); *. Correlation is significant at the 0.05 level (2-tailed).

A**CK**

	aadA	CTX	TEM	cmlA	floR	sul1	sul2	int1	uidA	TDS	pH	EC	TSS	
aadA	1.00	0.98 **	0.98 **	0.97 **	-0.18	0.71	0.93 **	0.34	0.97 **	0.72	-0.92 **	0.73	-0.96 **	
CTX		1.00	1.00	1.00	-0.13	0.54	0.91 *	0.14	1.00	0.57	-0.83 *	0.59	-0.92 **	
TEM			1.00	1.00	-0.07	0.55	0.94 **	0.13	1.00	0.56	-0.82 *	0.58	-0.91 *	
cmlA				1.00	-0.12	0.51	0.91 *	0.10	1.00	0.55	-0.81 *	0.57	-0.92 **	
floR					1.00	-0.28	0.14	-0.39	-0.07	-0.55	0.44	-0.56	0.45	
sul1						1.00	0.67	0.90 *	0.52	0.92 **	-0.85	0.91 *	-0.69	
sul2							1.00	0.27	0.93	0.55	-0.77	0.57	-0.80	
int1								1.00	0.10	0.84	-0.61	0.82	-0.38	
uidA									1.00	0.53	-0.80	0.55	-0.90	
NO3										0.73	-0.44	0.70	-0.19	
TDS											1.00	-0.93	1.00	
pH												1.00	-0.94 **	
EC													1.00	-0.82 *
TSS														1.00

B**WW**

	aadA	CTX	TEM	cmlA	floR	sul1	sul2	int1	uidA	TDS	pH	EC	TSS	
aadA	1.00	0.99 **	0.99 **	0.96 **	0.17	0.42	0.42	0.67	0.99 **	0.60	-0.84 *	0.60	-0.93 **	
CTX		1.00	0.97 **	0.92 **	0.11	0.51	0.50	0.75	0.98 **	0.69	-0.88 *	0.69	-0.95 **	
TEM			1.00	0.98 **	0.12	0.37	0.37	0.62	1.00	0.55	-0.81	0.55	-0.89 *	
cmlA				1.00	0.22	0.29	0.29	0.56	0.98 **	0.47	-0.74	0.47	-0.87 *	
floR					1.00	-0.26	-0.22	-0.11	0.13	-0.18	0.20	-0.17	-0.23	
sul1						1.00	0.97 **	0.95 **	0.36	0.98 **	-0.83 *	0.98 **	-0.64	
sul2							1.00	0.91 *	0.36	0.95	-0.82	0.95	-0.59	
int1								1.00	0.62	0.99 **	-0.93 **	0.99 **	-0.84 *	
uidA									1.00	0.54	-0.81	0.54	-0.89 *	
NO3										0.73	-0.45	0.73	-0.17	
TDS											1.00	-0.91 *	1.00	
pH												1.00	-0.91 *	
Tempe													0.13	
EC													1.00	
TSS														1.00

Figure S2. Correlation of the study genes with physicochemical water quality parameters in the multi-species cultures.

**Correlation is significant at the 0.01 level (2-tailed); *Correlation is significant at the 0.05 level (2-tailed)