Supplementary Materials - Figure

Figure S1. Time-course of influence of sub-inhibitory pinocembrin (16 µg/mL; 0.25 × MIC) on intracellular reactive oxygen species in *Campylobacter jejuni* NCTC 11168ΔcmeR.

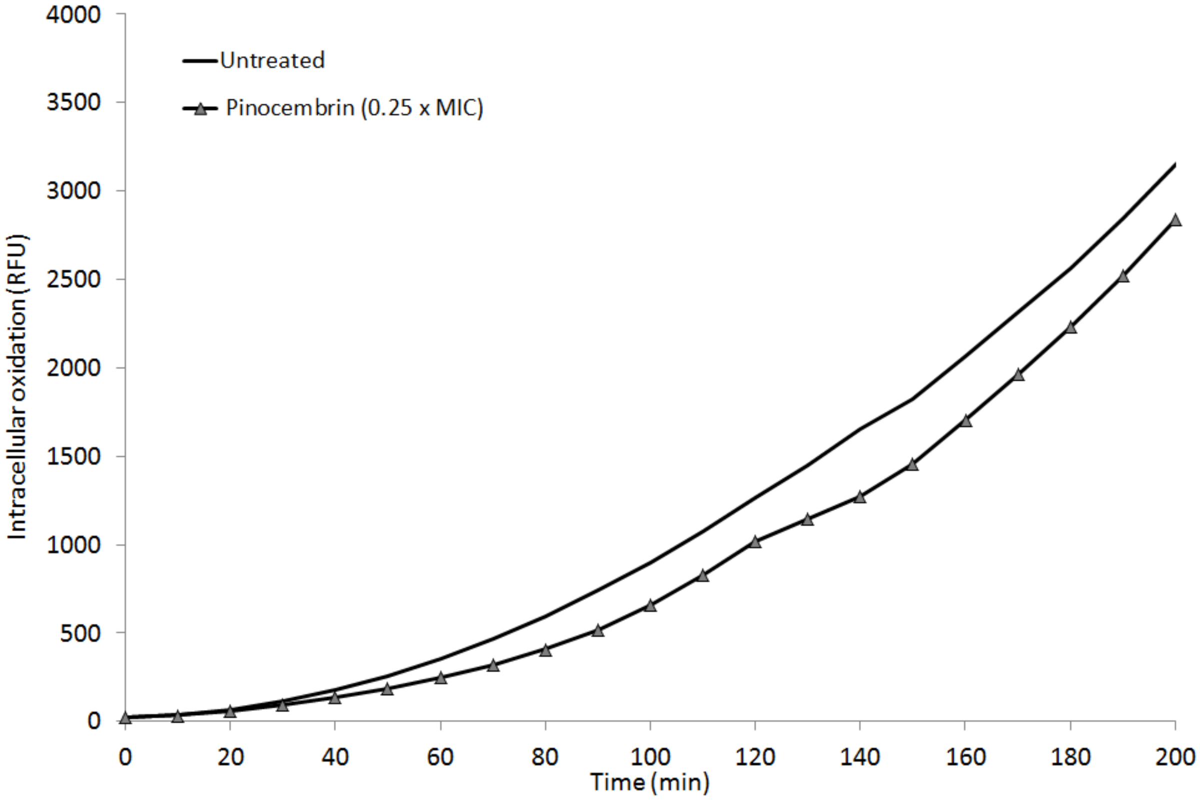

Figure S1

Supplementary Materials - Tables

Table S1. Primers of the genes for qRT-PCR confirmation used in this study.

Target	**Forward primer (5’-3’)**	**Reverse primer (5’-3’)**
nrfH	AAG TCA AAG GCG AAC CAA GA	CCT AAA CCA CTT TGC GCT TT
aspA	TGG GGA ATT GGA AAT CTC TG	CCC TAA CAA AGC GAG GAA AA
cstA	GTT GCT ATG GTG GCG ATT TT	CCC CAT GGA GAT TCT GCT AA
rpsD	TGC TTG GGT TGA TGT TGA AA	CCT CAA CCG GAA TGA CAA CT
nuoM	TGG GCA CCTA AGG TTT ATG C	CAC ACT CGC ATC TGG AAA AA
cfbpA	AAG CAT GGG CTA AAG GTG TG	AAA TTT AGC TTC GCC TGC AA
flaB	TAG CGC AGG AAG TGG ATT TT	TAG CGT TTG CAA AAC CTG TG
ahpC	CTG CTC CAG CGG TAT TAG GA	TAC CGC TCC TTT TGG ACC TA
flgD	TAT GCA AAA ATG GCT GGA CA	TGA ACC GCT TCC TCC AGT AG
sodB	TGG TTC AGG ATG GTT TTG GT	TGG TGT AGC TGC GTT TGA AG

Table S2. Antimicrobial activity of ciprofloxacin (CIP; 5 µg), erythromycin (ERY 15 µg) and pinocembrin (Pc 100 µg, 50 µg, 25 µg and 12.5 µg) against *C. jejuni* NCTC 11168 and its efflux pump knockout mutant strains lacking functional genes for efflux pumps CmeABC (Δ*cmeB*) and CmeDEF (Δ*cmeF*), and the efflux pump repressor CmeR (Δ*cmeR*), presented as inhibition zone diameter in mm.

		Inhibition zone diameter (mm)					
Method	**Strain**	**CIP**	**ERY**	**Pc 100**	**Pc 50**	**Pc 25**	**Pc 12.5**
Agar-well diffusion (Well diameter 4 mm)	11168	38	44	16	14	10	6
	Δ*cmeB*	36	45	29	27	21	9
	Δ*cmeF*	36	41	14	12	8	6
	Δ*cmeR*	35	38	17	15	11	8
Disc diffusion (Disc diameter 7 mm)	11168	34	40	13	11	9	7*
	Δ*cmeB*	39	39	25	18	15	8
	Δ*cmeF*	33	38	15	11	9	7*
	Δ*cmeR*	33	35	14	12	10	7*

*No inhibition zone

Table S3. Differentially expressed genes after treatment of *C. jejuni* 11168 Δ*cmeR* with pinocembrin (0.25 x MIC).

Gene ID and Functional Category			***P*-value**	***Q*-value**	***n*-Fold change**	
					Microarray	**qRT-PCR**
Cj0414		putative oxidoreductase subunit	0.0000	0.0000	9.04	NT
Cj0415		putative GMC oxidoreductase subunit	0.0000	0.0000	7.48	NT
Cj0334	*ahpC*	alkyl hydroperoxide reductase	0.0000	0.0000	6.13	1.8
Cj1153		putative periplasmic cytochrome C	0.0000	0.0003	4.16	NT
Cj0169	*sodB*	superoxide dismutase (Fe)	0.0000	0.0002	3.81	2.7
Cj1388		putative endoribonuclease L-PSP	0.0008	0.0058	3.73	NT
Cj0779	*tpx*	thiol peroxidase	0.0000	0.0003	3.36	NT
Cj1506c		putative MCP-type signal transduction protein	0.0000	0.0003	2.98	NT
Cj0420		putative periplasmic protein	0.0001	0.0016	2.97	NT
Cj0239c		NifU protein homolog	0.0000	0.0003	2.79	NT
Cj0236c		putative integral membrane protein	0.0000	0.0008	2.76	NT
Cj0416		hypothetical protein	0.0006	0.0051	2.76	NT
Cj0379c		putative molybdenum containing oxidoreductase	0.0060	0.0181	2.75	NT
Cj1338c	*flaB*	flagellin	0.0001	0.0019	2.70	1.1
Cj0515		putative periplasmic protein	0.0529	0.0735	2.65	NT
Cj0041	*fliK*	putative flagellar hook-length control protein	0.0050	0.0162	2.64	NT
Cj0987c		putative MFS (Major Facilitator Superfamily)	0.0000	0.0004	2.51	NT

Cj0378c		putative ferric reductase-like transmembrane	0.0000	0.0009	2.48	NT
Cj0725c		molybdopterin biosynthesis protein	0.0000	0.0009	2.43	NT
Cj0147c	*trxA*	thioredoxin	0.0053	0.0166	2.43	NT
Cj1224		putative iron-binding protein	0.0011	0.0071	2.39	NT
Cj0998c		putative periplasmic protein	0.0004	0.0040	2.38	NT
Cj1659	*p19*	periplasmic protein p19	0.0131	0.0298	2.37	NT
Cj0175c	*cfbpA*	putative iron-uptake ABC transport	0.0012	0.0073	2.34	1.1
Cj0040		hypothetical protein	0.0034	0.0130	2.24	NT
Cj1569c	*nuoK*	NADH dehydrogenase I chain K	0.0001	0.0018	2.24	NT
Cj0720c	*flaC*	flagellin	0.0016	0.0086	2.22	NT
Cj1566c	*nuoN*	NADH dehydrogenase I chain N	0.0000	0.0009	2.24	NT
Cj0417		hypothetical protein	0.0025	0.0110	2.23	NT
Cj1729c	*flgE2*	flagellar hook subunit protein	0.0091	0.0237	2.20	NT
Cj1339c	*flaA*	flagellin	0.0004	0.0038	2.19	NT
Cj0528c	*flgB*	flagellar basal-body rod protein	0.0001	0.0018	2.15	NT
Cj0898		putative histidine triad (HIT) family protein	0.1469	0.1453	2.13	NT
Cj0830		putative integral membrane protein	0.0388	0.0601	2.12	NT
Cj1228c	*htrA*	serine protease (protease DO)	0.0001	0.0018	2.11	NT
Cj1127c	*pglJ*	GalNAc transferase	0.0000	0.0010	2.10	NT
Cj1046c	*moeB*	putative molybdopterin biosynthesis protein	0.0001	0.0020	2.08	NT
Cj1534c		putative bacterioferritin	0.0138	0.0313	2.08	NT
Cj1626c		putative periplasmic protein	0.0004	0.0041	2.06	NT

Cj0912c	*cysM*	cysteine synthase	0.0019	0.0093	2.06	NT
Cj1572c	*nuoH*	NADH dehydrogenase I chain H	0.0165	0.0349	2.03	NT
Cj1568c	*nuoL*	NADH dehydrogenase I chain L	0.0004	0.0038	2.03	NT
Cj0538	*oorC*	OORC subunit of 2-oxoglutarate:acceptor	0.0002	0.0028	2.02	NT
Cj0440c		putative transcriptional regulator	0.0001	0.0014	2.00	NT
Cj0042	*flgD*	putative flagellar hook assembly protein	0.0008	0.0059	1.97	2.5
Cj1567c	*nuoM*	NADH dehydrogenase I chain M	0.0032	0.0128	-1.62	-1.1
Cj1594	*rpsD*	30S ribosomal protein S4	0.0001	0.0020	-1.76	-3.2
Cj0781	*napG*	putative ferredoxin	0.0009	0.0065	-2.02	NT
Cj0952c		putative HAMP containing membrane protein	0.0035	0.0132	-2.04	NT
Cj0903c		putative amino-acid transport protein	0.0011	0.0069	-2.05	NT
Cj0892c		putative periplasmic protein	0.0785	0.0958	-2.05	NT
Cj0633		putative periplasmic protein	0.0000	0.0004	-2.06	NT
Cj1593	*rpsK*	30S ribosomal protein S11	0.0000	0.0008	-2.06	NT
Cj0671	*dcuB*	anaerobic C4-dicarboxylate transporter	0.0019	0.0093	-2.13	NT
Cj0911		putative periplasmic protein	0.0075	0.0208	-2.17	NT
Cj1357c	*nrfA*	putative periplasmic cytochrome C	0.0004	0.0037	-2.18	NT
Cj0021c		putative fumarylacetoacetate (FAA) hydrolase	0.0005	0.0046	-2.24	NT
Cj0854c		putative periplasmic protein	0.0005	0.0046	-2.28	NT
Cj0073c		conserved hypothetical protein Cj0073c	0.0005	0.0043	-2.30	NT
Cj0074c		putative iron-sulfur protein	0.0000	0.0009	-2.31	NT
Cj0909		putative periplasmic protein	0.0008	0.0061	-2.33	NT

Cj0449c		conserved hypothetical protein	0.0001	0.0018	-2.33	NT
Cj0153c		putative rRNA methylase	0.0009	0.0063	-2.39	NT
Cj0473	*nusG*	transcription antitermination protein	0.0001	0.0018	-2.40	NT
Cj1592	*rpsM*	30S ribosomal protein S13	0.0108	0.0265	-2.48	NT
Cj0917c	*cstA*	putative integral membrane protein	0.0000	0.0012	-2.55	-5.5
Cj0910		putative periplasmic protein	0.0011	0.0070	-2.58	NT
Cj1358c	*nrfH*	putative periplasmic cytochrome C	0.0000	0.0004	-2.73	-6.7
Cj0893c	*rpsA*	30S ribosomal protein S1	0.0000	0.0004	-2.74	NT
Cj0471	*rpmG*	50S ribosomal protein L33	0.0001	0.0014	-2.87	NT
Cj0358		putative cytochrome C551 peroxidase	0.0022	0.0102	-2.89	NT
Cj0087	*aspA*	aspartate ammonia-lyase	0.0002	0.0023	-3.05	-5.8
Cj0864		putative periplasmic protein	0.0003	0.0033	-3.14	NT

NT, not tested