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Supplementary Materials: The Novel ncRNA OsiR Positively Regulates Expression of *katE2* and Is Required for Oxidative Stress Tolerance in *Deinococcus radiodurans*

1. Supplementary Tables

Table S1. Distribution of homologous genes of *katE1* and *katE2* in *Deinococcus* species.

Species	***katE1*** **of homologous genes (number)**	**Identity (%)**	***katE2*** **of homologous genes (number)**	**Identity (%)**
D. radiodurans	1		1	
D. wulumuqiensis	1	96.83	1	88.39
D. reticulitermitis	1	87.64	1	65.96
D. sp K2S05-167			1	61.52
D. sp.FeSDHB5-19			1	67.08
D. proteolyticus	1	85.91	1	58.70
D. radiophilus	1	84.62	1	59.08
D. ficus			1	60.40
D. irradiatisoli	1	78.22	1	58.36
D. sp H1			1	59.12
D. sp S14-83			1	58.79
D. sp leaf326	1	82.90		
D. sp S9	1	78.07		
D. geothermalis	1	78.07		
D. phoenicis	1	77.97		
D. sp YIM 77859	1	78.10		
D. metallitatus	1	76.65		
D. deserti	1	74.71		
D. aerius	1	74.95		
D. apachensis	1	72.62		
D. planocerae	1	72.61		
D. sp RL	1	77.71		
D. murrayi	1	77.71		
D. yavapaiensis	1	71.57		

Red indicates that the homologous genes of *katE1* and *katE2* coexist in the same bacteria.

Table S2. Primers used in this study.

Primer Name	Sequences (5'–3')	Purpose
osiR-P1	ACCGAGGACCTGATCGAGGCTGC	Δ*osiR* deletion mutant consturction
osiR-P2	GAGTTTTTCTAATCATTTGCCCACGCAGAAGTTG	
osiR-P3	GTGGGCAAATGATTAGAAAAACTCATCGAGCATCAAATG	
osiR-P4	GCTAAAATACAGACCATGGAGACCGAGGGCCCTTGACATT	
osiR-P5	CCTCGGTCTCCATGGTCTGTATTTTAGCGTGTTAG	
osiR-P6	GTTCCGTCGCGCCCGTCACTTCG	
katE2-P1	CGCCGCGCTCGCCATCAGTG	Δ*katE2* deletion mutant consturction
katE2-P2	CAAGGGCCCTCGGTCTCCATGCAGAGCGCGGCCTTTTCGGGA	
katE2-P3	TCCCGAAAAGGCCGCGCTCTGCATGGAGACCGAGGGCCCTTG	
katE2-P4	GCCACCTTGAGGACTAATGGTTTAGAAAAACTCATCGAGC	
katE2-P5	GCTCGATGAGTTTTTCTAAACCATTAGTCCTCAAGGTGGC	
katE2-P6	TGACAGACAGCGCCTCGGCG	
qosiR-F	GTCTGACCTCGACCTCACCAC	qRT-PCR
qosiR-R	GGCAAATGATGCGGCCTCAAGC	
qkatE2-F	TGGGCAAGATGGTCCTCG	
qkatE2-R	CAGCGGGTCGTTGGTGA	
qkatE1-F		
qkatE1-R		
qDR_1279-F		
qDR_1279-R	ACCAACATCCAGTCGCAG	
qDR_1546-F	CCTTCACCCTGGTCGTTG	
qDR_1546-R	CCGCACGATGGAAATTCACC	
qDR_A0202-F	CAGAACATGCTGTGGTTGGC	
	CTTTAGCCCCAGTCCCATCG	
qDR_A0202-R	ACCGTAATCATCGGCAGGTC	
	CTGAACCGCAGTCTGGTCAT	
qDR_A0301-F	AAGCCTTTCTTCGCGTCGTA	
	TCGACAAGGCCGAGTGTTTT	
qDR_A0301-R	GAGGCCGATGTCTTCGTTGA	
	GACGATATTCAGGCGACCGT	
qDR_A0145-F	CCACTTTGAACGACTCGGGA	
qDR_A0145-R		
q16S-F	ATTCCTGGTGTAGCGGTG	
q16S-R	CATCGTTTAGGGTGTGGAC	
5S-NP	Dig-5'GTATCATTGGCGCTGCTGTGTTTCACGACCCAGTT	Northern blot
OsiR-NP	Dig-5'TGTATTCGTCTGACCTCGACCTCACCACCAA	
GSP1	5'-GACGTGCAGGAAGATGTGGTGGACGCTGTGTTTC-3'	5'RACE
GSP2	5'-GTGTTTCGCAACTTCTGCGTGGGCAAATGATGC-3'	
GSP3	5'-CAAGCGCTCCGGCGCAGGCGGCGTTCCAGCCAG-3'	

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Table A3. Synthesized ssRNA oligonucleotide derivatives for MST.

Name	Sequence (5'–3')	Relevant Characteristics
OsiR-wt	AUGUAUUCGUCUGACCUCGACCUCACCACCAAAGCCCUCAUGCGCGAAC AGGCCCUUCACGAGCCGCAGGCCAUGACCUCAUGGGAAGCUCAUGACCG CCGCGAAAGCCGCAGCGUGCGCCGCGCCCUGAACUGGCUGGAACGCCGC CUGCGCCGGAGCGCUUGAGGCCGCAUCAUUUGCCCACGCAGAAGUUGCG AAACACAGCGUCCACCACAUCUUCCUGCACGUCCUGACCGGUGAGCUCG GCCAGG	wild type (251 bp), interaction with *katE2*-wt
OsiR-mut	AUGUAUUCGUCUGACCUCGACCUCACCACCAAAGCCCUCAUGCGCGAAC AGGCCCUUCACGAGCCGCAGGCCAUGACCUCAUGGGAAGCUCAUGACCG CCGCGAAAGCCGCAGCGUGCGCCGCGCCCUGAACUGGCUGGAACGCCGC CUGCGCCGGAGCGCUUGAGGCCGCAUCAUUUGCCCACGCAGAAGUUGCG AAACACACGCAGGUGGAGUAGAACGACGACGUCCUGACCGGUGAGCUCG GCCAGG	mismatch mutation (251 bp), no binding affinity with *katE2*-wt
OsiR-com	AUGUAUUCGUCUGACCUCGACCUCACCACCAAAGCCCUCAUGCGCGAAC AGGCCCUUCACGAGCCGCAGGCCAUGACCUCAUGGGAAGCUCAUGACCG CCGCGAAAGCCGCAGCGUGCGCCGCGCCCUGAACUGGCUGGAACGCCGC CUGCGCCGGAGCGCUUGAGGCCGCAUCAUUUGCCCACGCAGAAGUUGCG AAACACAGCGUCCACCGCAUCUUCCUGCACGUCCUGACCGGUGAGCUCG GCCAGG	compensatory mutation (251 bp), stronger interaction with *katE2*-wt
katE2-wt	UGCUCGGCGUCAAGAGCCUGGUGUGGGACGAAGCGCAGAAGAUUGCCG GUAAGGACGCCGACUUCCACCG	wild type (70 bp), interaction with osiR-wt
N.	UGCUCGGCGUCAAGAGCCUGGUGUGGGACGAAGCGCGUCCACCACAUCU UCCUGCCTACGACUUCCACC	ompletely mismatched (70 bp), no binding with osiR-wt

wt: wild type; mut: mutation; com: supplementary mutation; N.C: negative control. The base pairing region of OsiR with *katE2* mRNA is shown in red. Point mutations introduced into synthesized oligonucleotide derivatives are shown in green.

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2. Supplementary Figure

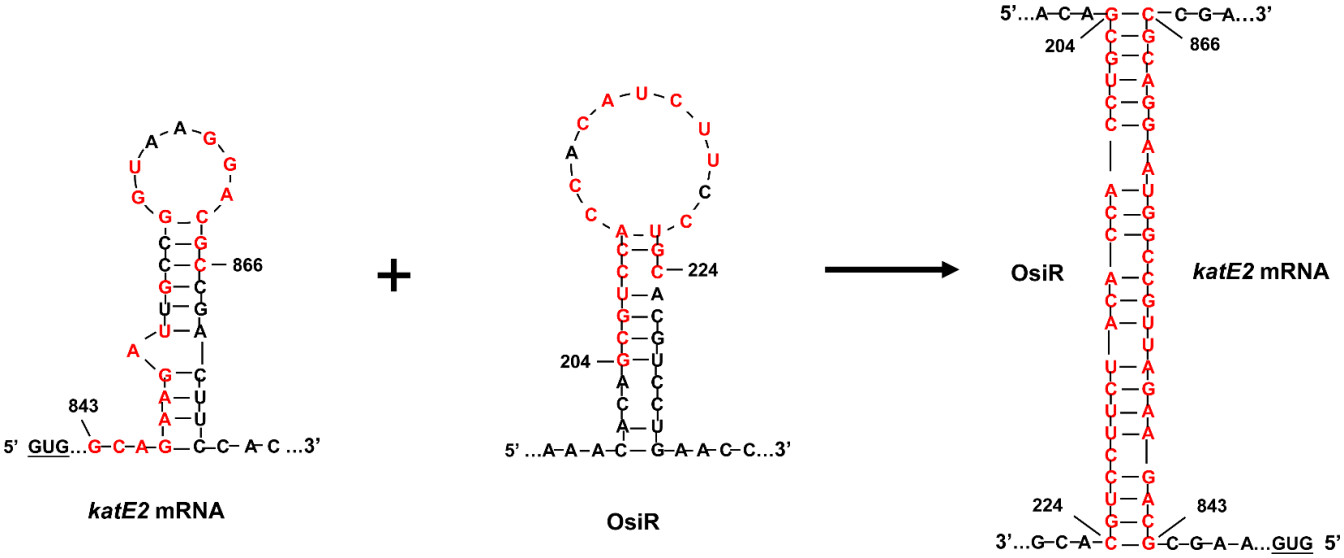

Figure S1. Schematic of the interaction between OsiR and *katE2* mRNA. The secondary structure of the *katE2* mRNA contains a putative hairpin structure (nucleotides 846–873), which includes the OsiR binding site, presumably forming an inhibitory structure that affects translation efficiency. The binding of OsiR and *katE2* mRNA promotes the ribosome binding and progression.