

Supplementary Material

Deionococcus proteotlycius* Genomic Library Exploration Enhances Oxidative Stress Resistance and Poly-3-hydroxybutyrate Production in Recombinant *Escherichia coli

Seul-Ki Yang ^{1,2}, Soyoung Jeong ^{1,3}, Inwoo Baek ¹, Jong-il Choi ⁴, Sangyong Lim ^{1,5} and Jong-Hyun Jung ^{1,*}

¹ Radiation Biotechnology Division, Korea Atomic Energy Research Institute, Jeongseup 56212, Republic of Korea; wisdomyang@kaeri.re.kr (S.-K.Y.); soyoung@kaeri.re.kr (S.J.);

inwoobaek@kaeri.re.kr (I.B.); saylim@kaeri.re.kr (S.L.)

² Graduate School of Biotechnology and Institute of Life Science and Resources, Kyung Hee University, Yongin 17104, Republic of Korea

³ Department of Food and Animal Biotechnology, Seoul National University, Seoul 08826, Republic of Korea

⁴ Department of Biotechnology and Bioengineering, Chonnam National University, Gwangju 61186, Republic of Korea; choiji01@chonnam.ac.kr

⁵ Department of Radiation Science and Technology, University of Science and Technology, Daejeon 34113, Republic of Korea

* Correspondence: jungjh83@kaeri.re.kr; Tel: +82-63-570-3203; Fax: +82-63-570-3238

Keywords: *Deinococcus proteolyticus*, oxidative stress, response regulator, PHB

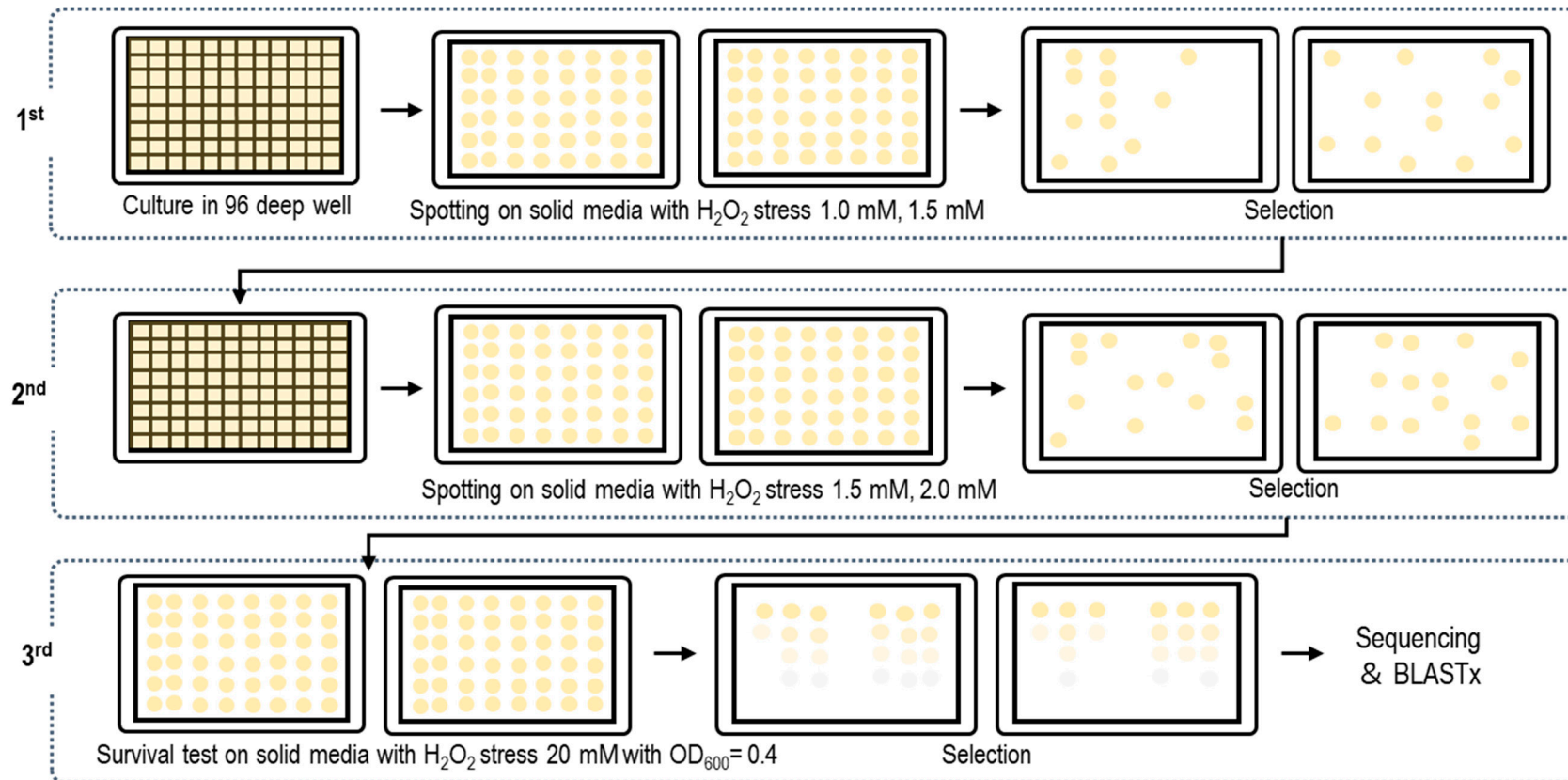
Supple Table S1. List of quantitative real-time PCR primers in this study

Pathway	Gene	Forward primer	Reverse primer
Internal control	<i>polA</i>	GCTGAACGTGCAGCCATTAA	CAATCATCGCCCGTTTGATAA
	<i>dnaA</i>	TGGCGAAAGAGCTGACTAACC	ACGGCAGGCATGAAGCA
Oxidative stress related gene	<i>katE</i>	CAATTCCACAAGGGCAAAGT	CTTCCCTTCGGCATTAAATCA
	<i>katG</i>	GCATCTGCTTCTACCTTCCG	CTCAACACCAACCACACCAG
	<i>dps</i>	AAACAAGCGCACTGGAACAT	CTGCACTGCACGTTCTGC
	<i>ahpC</i>	ACTGCAGAAACTGGGCGTAG	GTTGTCGAAGTTACGGGTCAG
	<i>sodA</i>	GCTGAAAGGCGATAAACTGG	ACACATCCAGGCCCATATC
Sigma factors	<i>rpoS</i>	CGCCGCCGGATGATC	CAGACCACGATTGCCATAACG
Oxidative stress regulator	<i>oxyR</i>	GAAGCACAGACCCACCAGTT	CAAACAACGGCACTTCAATG
	<i>soxS</i>	TACTTGCAACGAATGTTCCG	ACATAACCCAGGTCCATTGC
Pentose phosphate pathway Glycolysis	<i>ptsG</i>	ACTGGCGTTGTGCTGTCCTT	GGTAAGCAGCCCACTGAGAGA
	<i>zwf</i>	GGCGCTGCGTTTTGCTAA	TTCTGCCACGGTAATCTCAACA
	<i>pykA</i>	GCGCTGACCGAAAAAGACAA	CAGCCAGGTAATCTACGCCAAT
	<i>pykF</i>	CGAGTTCAACAATGACAACCGTAA	ATCAGCGGAGCATCCAGTTT
	<i>aceE</i>	CCAAAGGCAAAGCGACAGTAAT	CCTGGTGCGCGATGTTTT
TCA cycle	<i>icdA</i>	AAGTTCACCGAAGGAGCGTTTA	GCCACCGTCGATCAGTTCA
	<i>sucA</i>	GTCTCGCGTTGCCAAGATTTAT	GTGGCGTAAGCGAGGTTTTTC
	<i>mdh</i>	AGGCGCTTGCACTACTGTTAAAA	CGGGAGTCACTGGAGCGATA
	<i>sdhC</i>	CACATGATGATGGATTTTGGCTAT	GCACGACAGTAATAACAAAGGA GATT
Gluconeogenesis and anaplerosis	<i>pps</i>	CCTCCCTGGGTGAAATGATTACTA	GGCGGTTGTGGCGAAA
	<i>ppc</i>	GGAAGAGAACCTCGGCTACAAA	CCGCCCATCCACGAAGT
	<i>pck</i>	CGTCTTTCCGTCCGTTTCAT	GCTCGGGCGAATAAACATGT
	<i>maeB</i>	GAAGAGCTGGCGGAGATCAC	GCGGCTCAATACCAAAACGA
	<i>sfcA</i>	TGCGGTTGGCAAAATGG	AGGGCTTCGGCAGAGGTT
Glyoxylate shunt	<i>aceA</i>	TGCACGGTGAGTCGAAAAAA	TAGACTGCTTCAATACCCGCTTT
	<i>aceB</i>	TGGCGTGGTGAGGCAAT	GGAAGAAATAGAGCGCAAAATCA

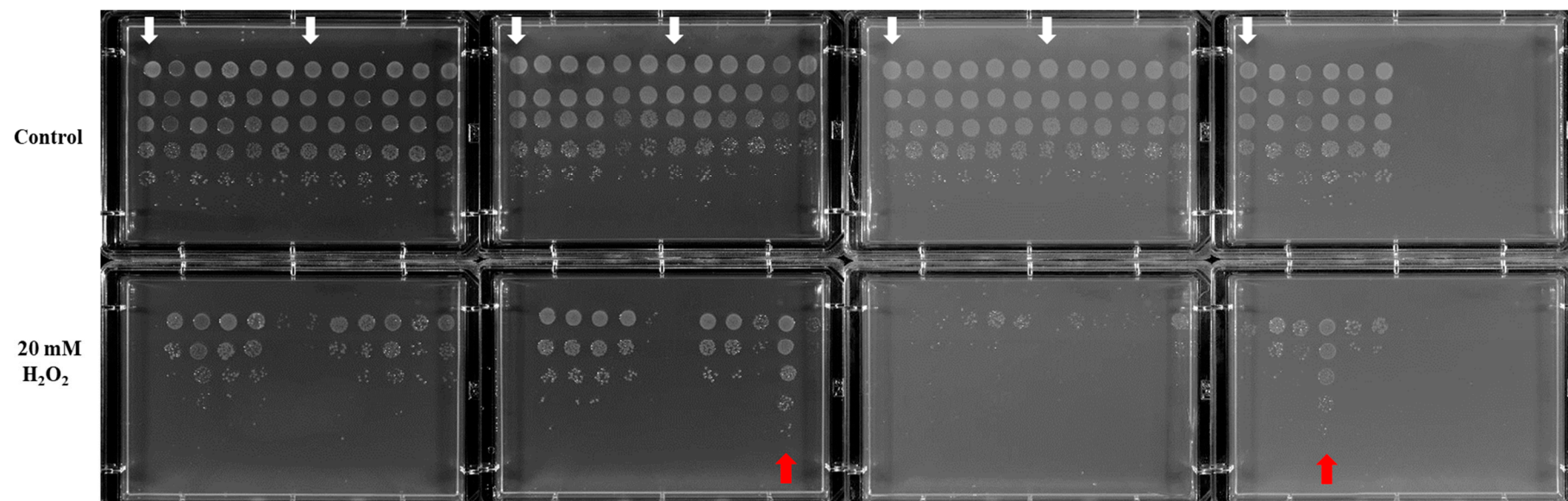
Supple Table S2. Genetic information of the neighbor genes of *deipr_0871* and its homolog, *dr_0891*

The list of genes in <i>D. proteolyticus</i>		
locus_tag	Location	Product
Deipr_0866	918064..917162	hypothetical protein
Deipr_0867	919101..918133	NUDIX hydrolase
Deipr_0868	919285..919527	cold-shock DNA-binding domain protein
Deipr_0869	921102..919723	Methylenetetrahydrofolate--tRNA-(uracil-5-)-methyltransferase
Deipr_0870	921241..922314	integral membrane sensor signal transduction histidine kinase
Deipr_0871	922311..923012	two component transcriptional regulator, LuxR family
Deipr_0872	923052..923813	protein of unknown function DUF124
Deipr_0873	924924..923878	thioredoxin reductase
Deipr_0874	925053..925643	Holliday junction ATP-dependent DNA helicase ruvA

The list of genes in <i>D. radiodurans</i>		
locus_tag	Location	Product
DR_0888	897508..898269	hypothetical protein
DR_0889	898277..898627	hypothetical protein
DR_0890	898628..899065	conserved hypothetical protein
DR_0891	899727..899143	DNA-binding response regulator
DR_0892	900833..899724	sensor histidine kinase
DR_0893	901689..900994	conserved hypothetical protein
DR_0894	903279..901774	hypothetical protein
DR_0895	903383..903769	hypothetical protein
DR_0896	903759..904532	ribosomal large subunit pseudouridine synthase B



Supple Fig. S1. Screening of positive clones by hydrogen peroxide. *E.coli* (XL1-Blue) containing recombinant DNA fragments were incubated in liquid medium with 50 $\mu\text{g}/\text{mL}$ kanamycin and spotting on 1.0, 1.5, and 2.0 mM H_2O_2 in 1st, 2nd screening step, 20 mM H_2O_2 for 1 hr in 3rd screening.



Supple Fig. S2. 3rd screening selection. The white arrows indicate *E.coli* harboring empty vector as control; The red arrows indicate selected *E.coli* harboring *D. proteolyticus* genes with outstanding oxidative stress resistance. Adjusting cell OD₆₀₀= 0.4

TCS HK	TCS-RR	DNA binding domain
Deipr_0128	Deipr_0129	OmpR family
Deipr_0376	Deipr_0377	-
Deipr_0799	Deipr_0800	NarL/FixJ family
Deipr_0870	Deipr_0871	NarL/FixJ family
Deipr_1062	Deipr_1061	-
Deipr_1165	Deipr_1167	NarL/FixJ family
Deipr_1504	Deipr_1503	OmpR family
Deipr_1922	Deipr_1923	OmpR family
Deipr_1967	Deipr_1968	OmpR family
Deipr_2675	Deipr_2674	OmpR family
Deipr_2694	Deipr_2693	OmpR family

HK : Histidine kinase

RR : Response regulator



Core gene (>50%)



Accessory gene (<50%)

Orphan HK	Additional domain
Deipr_0252	GAF domain
Deipr_0865	-
Deipr_1801	PAS/PAC domain
Deipr_2217	GAF domain
Deipr_2254	-
Deipr_2523	PAS/PAC domain

Orphan RR	Domain
Deipr_0059	-
Deipr_0147	OmpR family
Deipr_0158	OmpR family
Deipr_0764	wHTH and BTAD domain
Deipr_1307	OmpR family
Deipr_2630	-

Supple Fig. S3. Core and accessory analysis of two component system, orphan histidine kinase and orphan response regulators in *D. proteolyticus*. Core and accessory genes were determined based on the homology (50%) and functional domains were analyzed using interpro database.