

Quorum sensing regulator OpaR directly represses seven protease genes in *Vibrio parahaemolyticus*

Supplementary materials:

Table S1. Bacterial strains and plasmids used in this study.

Table S2. Primers used in this study.

Figure S1. Expression levels of FLAG-OpaR at different growth phases.

Table S1 Bacterial strains and plasmids used in this study

Strain or plasmid	Description ^a	Reference
Strain		
<i>V. parahaemolyticus</i> no. 93	<i>tdh⁻ trh⁻</i>	(Yu & Lee, 1999)
<i>ΔopaR</i>	no.93 <i>ΔopaR</i>	(Chang & Lee, 2018)
FLAG- <i>opaR</i> /VP93	no.93 FLAG- <i>opaR</i>	This study
pSAluxAB/VP93	no.93 with pSAluxAB	This study
pSAluxAB/ <i>ΔopaR</i>	<i>ΔopaR</i> with pSAluxAB	This study
pSAluxAB- <i>lytM</i> promoter /VP93	no.93 with pSAluxAB- <i>lytM</i> promoter	This study
pSAluxAB- <i>lytM</i> promoter / <i>ΔopaR</i>	<i>ΔopaR</i> with pSAluxAB- <i>lytM</i> promoter	This study
pSAluxAB- <i>mcp02</i> promoter /VP93	no.93 with pSAluxAB- <i>mcp02</i> promoter	This study
pSAluxAB- <i>mcp02</i> promoter / <i>ΔopaR</i>	<i>ΔopaR</i> with pSAluxAB- <i>mcp02</i> promoter	This study
pSAluxAB- <i>m6 protease</i> promoter /VP93	no.93 with pSAluxAB- <i>m6 protease</i> promoter	This study
pSAluxAB- <i>m6 protease</i> promoter / <i>ΔopaR</i>	<i>ΔopaR</i> with pSAluxAB- <i>m6 protease</i> promoter	This study
pSAluxAB- <i>serine protease</i> promoter /VP93	no.93 with pSAluxAB- <i>serine protease</i> promoter	This study
pSAluxAB- <i>serine protease</i> promoter / <i>ΔopaR</i>	<i>ΔopaR</i> with pSAluxAB- <i>serine protease</i> promoter	This study
pSAluxAB- <i>degS</i> promoter /VP93	no.93 with pSAluxAB- <i>degS</i> promoter	This study
pSAluxAB- <i>degS</i> promoter / <i>ΔopaR</i>	<i>ΔopaR</i> with pSAluxAB- <i>degS</i> promoter	This study
pSAluxAB- <i>protease II</i> promoter /VP93	no.93 with pSAluxAB- <i>protease II</i> promoter	This study
pSAluxAB- <i>protease II</i> promoter / <i>ΔopaR</i>	<i>ΔopaR</i> with pSAluxAB- <i>protease II</i> promoter	This study
pSAluxAB- <i>periplasmic protease</i> 3'region /VP93	no.93 with pSAluxAB- <i>periplasmic protease</i> 3'region	This study
pSAluxAB- <i>periplasmic protease</i> 3'region / <i>ΔopaR</i>	<i>ΔopaR</i> with pSAluxAB- <i>periplasmic protease</i> 3'region	This study
<i>Escherichia coli</i>		
XL1-Blue	<i>recA1endA1 gyrA96 thi⁻¹ hsdR17 supE44 relA1 lac [F' proAB lacI^q Δ(lacZ) M15 Tn10 (Tet^r)], cloning host</i>	Stratagene
S17-1 <i>λpir</i>	<i>recA::RP4-2-Tc::Mu λ pir R6K</i>	(Delorenzo <i>et al.</i> , 1993)
BL21 (DE3)	F ⁻ <i>ompT gal dcm lon hsdS_B (r_B⁻ m_B⁻) λ (DE3) [lacI lacUV5-T7 gene 1 <i>ind1 sam7 nin5</i>])</i>	Novagen
XL1-Blue/pDS132-FLAG- <i>opaR</i>	XL1-Blue with pDS132-FLAG- <i>opaR</i> plasmid	This study
S17-1 <i>λpir</i> /pDS132-FLAG- <i>opaR</i>	S17-1 <i>λpir</i> with pDS132-FLAG- <i>opaR</i> plasmid	This study
pSAluxAB- <i>lytM</i> promoter/ XL1-Blue	XL1-Blue with pSAluxAB- <i>lytM</i> promoter	This study
pSAluxAB- <i>mcp02</i> promoter / XL1-Blue	XL1-Blue with pSAluxAB- <i>mcp02</i> promoter	This study
pSAluxAB- <i>m6 protease</i> promoter / XL1-Blue	XL1-Blue with pSAluxAB- <i>m6 protease</i> promoter	This study
pSAluxAB- <i>serine protease</i> promoter / XL1-Blue	XL1-Blue with pSAluxAB- <i>serine protease</i> promoter	This study

pSAluxAB- <i>degS</i> promoter / XL1-Blue	XL1-Blue with pSAluxAB- <i>degS</i> promoter	This study
pSAluxAB- <i>protease II</i> promoter / XL1-Blue	XL1-Blue with pSAluxAB- <i>protease II</i> promoter	This study
pSAluxAB- <i>periplasmic protease</i> 3'region/ XL1-Blue	XL1-Blue with pSAluxAB- <i>periplasmic protease</i> 3'region	This study
pET28a- <i>opaR</i> / XL1-Blue	XL1-Blue with pET28a- <i>opaR</i>	This study
pET28a- <i>opaR</i> / BL21 (DE3)	BL21 (DE3) with pET28a- <i>opaR</i>	This study
Plasmids		
RBC TA Vector	cloning Vector, Amp ^r	RBC
pDS132	R6K ori, mobRP4, <i>cat</i> , <i>sacB</i> suicide vector for gene replacement, Cm ^r	(Philippe <i>et al.</i> , 2004)
pET28a	expression vector, Km ^r	Novagen
pSA19CP-MCS	<i>Vibrio parahaemolyticus</i> plasmid pSA19 holding MCS derived from pUC119, Cm ^r	(Nomura <i>et al.</i> , 2000)
pSAluxAB	pSA19CP vector with luxAB reporter gene, Cm ^r	This study
pDS132- <i>mopaR</i>	derivative of pDS132 for generating the <i>opaR</i> deletion mutant, Cm ^r	This study
pDS132-FLAG- <i>opaR</i>	derivative of pDS132 for generating the FLAG- <i>opaR</i> , Cm	This study
pSAluxAB- <i>lytM</i> promoter	pSAluxAB with 740 bp <i>lytM</i> promoter for luciferase assay, Cm ^r	This study
pSAluxAB- <i>mcp02</i> promoter	pSAluxAB with 622 bp <i>mcp02</i> promoter for luciferase assay, Cm ^r	This study
pSAluxAB- <i>m6 protease</i> promoter	pSAluxAB with 708 bp <i>m6 protease</i> promoter for luciferase assay, Cm ^r	This study
pSAluxAB- <i>serine protease</i> promoter	pSAluxAB with 679 bp <i>serine protease</i> promoter for luciferase assay, Cm ^r	This study
pSAluxAB- <i>degS</i> promoter	pSAluxAB with 552 bp <i>degS</i> promoter for luciferase assay, Cm ^r	This study
pSAluxAB- <i>protease II</i> promoter	pSAluxAB with 607 bp <i>protease II</i> promoter for luciferase assay, Cm ^r	This study
pSAluxAB- <i>periplasmic protease</i> 3'region	pSAluxAB with 509 bp <i>periplasmic protease</i> 3'region for luciferase assay, Cm ^r	This study
pET28a- <i>opaR</i>	pET28a containing <i>opaR</i> coding region, Km ^r	This study

^aAmp^r, ampicillin resistance; Cm^r, chloramphenicol resistance; Km^r, kanamycin resistance

References

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- Yu, M.S., and Lee, C.Y. (1999) Expression and characterization of the *prtV* gene encoding a collagenase from *Vibrio parahaemolyticus* in *Escherichia coli*. *Microbiol-Sgm* 145, 143-150.

Table S2 Primers used in this study

Primer name	Sequence (5' → 3')^a	Source
<i>Strain construction</i>		
Vp2516D-A2	<u>TAGCATGCT</u> TACCTGTATCGA	This study
Vp2516D-B1	GAGCTCTTTCAACTGTTGTTGCTTACGTTTAA	This study
Vp2516D-C1	GTAAGCAACAACAGTTGAAAGAGCTCGTGAG	This study
Vp2516D-D1	GTCTAGAAATCGACGAGCG	This study
VP2516-FLAG-A	<u>CATGCATGCGCTCTGTAATTTGTTTGCC</u>	This study
VP2516-FLAG-B	TTTATCGTCGTCATCTTTGTAATC (FLAG)	This study
	CATATCCATTTTCCTTGCC	
VP2516-FLAG-C	TTTATCGTCGTCATCTTTGTAATC (FLAG)	This study
	CATATCCATTTTCCTTGCC	
VP2516-FLAG-D	<u>GCTCTAGATT</u> CGTGTTCAAATCTGAGC	This study
VP2516-FLAG-F	GATTACAAAGATGACGACGAT AAA	This study
VP2516-FLAG-R	GATGCGCTCCACTCGAACC	This study
Vp2516D_ICF	GCAAACGATCACCTAATCT	This study
Vp2516D_ICR	GGGTCTAGCTGCTGAAGTA	This study
<i>ChIP-qPCR</i>		
VPAr01_132951_F	GCGGCAGGCCTAACACAT	This study
VPAr01_133200_R	CGCCTTGGTGAGCCCTTAC	This study
VP2762-ChIP-q-F	ATAATTGCGCAGCAAATAACC	This study
VP2762-ChIP-q-R	GCATGAAGTGGAATACTAGGACAA	This study
VPA0606-ChIP-q-F	CTGTAAGTGAAGAATAATACCGAATG	This study
VPA0606-ChIP-q-R	AAGTGGTTAAAGCGAATGATC	This study
<i>qRT-PCR</i>		
VPAr01_443_F	ACTTTCAGTCGTGAGGAAGG	This study
VPAr01_701_R	CACCGCTACACCTGAAATTC	This study
VP2516_279_F	CGCAAACATCACCAATGCGA	This study
VP2516_531_R	TGCTTGTACGAACAGCGAGT	This study
VPA1649_323_F	CGAATGTACGAACGCTGATG	This study
VPA1649_563_R	AAAGAGTGCTGTAGCCTCAG	This study
VPA0755_1106_F	CTGGCGGTATTAATGAAGCG	This study
VPA0755_1373_R	GTATTTTCAGTGCCCCAACCC	This study
VP0907_815_F	GGCCACACAAATTCTCTCAC	This study
VP0907_1074_R	CATGTTAACGGGCGTATCAC	This study
VPA0449_421_F	AATGAAGGTCTCGGTGGAAG	This study
VPA0449_681_R	AATGCTACTCACGCTATCCC	This study
VP0432_299_F	TAGCTCAAGCAGACCAAGTC	This study
VP0432_535_R	GACCTGTCGCGGAAATAATG	This study
VPA1467_897_F	GATACTGCCATTGGAACACG	This study
VPA1467_1130_R	GGAACAGAAACACCATCGTG	This study
VPA2032_786_F	CAAAGACTTCATCCGTGAGC	This study
VPA2032_1016_R	CTGTCAGCGACTTTACTTGC	This study
<i>LuxAB assay</i>		
VP0907-luxAB-F1	TGCAGGTTTGACGGCAGAGC	This study
VP0907-luxAB-R1	TCAGGGGTGTCTGAATTACGCT	This study
VPA1649-luxAB-F1	CGGTTCGTAAATGCGTTTTGC	This study
VPA1649-luxAB-R1	CCTCTCGCTCTGAAAGGTCGTT	This study
VPA0755-luxAB-F1	GAAGGTCGCGCGGTCAAAA	This study

VPA0755-luxAB-R1	TGGCTCTAATCGCTCCAACTGA	This study
VP0432-luxAB-F1	CGGAGCTTCGCTCTATTGTTGA	This study
VP0432-luxAB-R1	CAGAACCTAGCCCTTGTGTCTGA	This study
VPA0449-luxAB-F1	ATGCGTTTTTTGAGCGGTTGG	This study
VPA0449-luxAB-R1	TCTGCTCTGACGCATCAACTCC	This study
VPA2032-luxAB-F1	TGATGATCTTGTGTTGCTGCG	This study
VPA2032-luxAB-R1	CCAACTTTTTTCGCCAGCAACAT	This study
VPA1467-luxAB-F1	ACAAAAACGTATCGGTGGGTGA	This study
VPA1467-luxAB-R1	TGCGCCAGCAGATCTTGTGT	This study
PSA19CP-F	TGCGTTCGTGTCTTTGAATC	This study
<i>His-OpaR expression</i>		
VP2516-EXF	GCGGGATCCATGGACTCAATTGCAAAG	This study
VP2516-EXR	GCGAAGCTTTTAGTGTTTCGCGATTGTAG	This study
<i>Footprinting assay</i>		
VP0907-footprint-F-6FAM	TGCAGGTTTGACGGCAGAGC	This study
VP0907- footprint-R	TCAGGGGTGTCTGAATTACGCT	This study
VP0907-footprint-F	TGCAGGTTTGACGGCAGAGC	This study
VP0907- footprint-R-6FAM	TCAGGGGTGTCTGAATTACGCT	This study
VPA1649-footprint-F-6FAM	CGGTTCGTAAATGCGTTTTGC	This study
VPA1649- footprint-R	CCTCTCGCTCTGAAAGGTCGTT	This study
VPA1649-footprint-F	CGGTTCGTAAATGCGTTTTGC	This study
VPA1649- footprint-R-6FAM	CCTCTCGCTCTGAAAGGTCGTT	This study
VPA0755- footprint-F-6FAM	GAAGGTCGCGCGGTCAAAA	This study
VPA0755- footprint-R	TGGCTCTAATCGCTCCAACTGA	This study
VPA0755- footprint-F	GAAGGTCGCGCGGTCAAAA	This study
VPA0755- footprint-R-6FAM	TGGCTCTAATCGCTCCAACTGA	This study
VPA0449- footprint-F-6FAM	ATGCGTTTTTTGAGCGGTTGG	This study
VPA0449- footprint-R	TCTGCTCTGACGCATCAACTCC	This study
VPA0449- footprint-F	ATGCGTTTTTTGAGCGGTTGG	This study
VPA0449- footprint-R-6FAM	TCTGCTCTGACGCATCAACTCC	This study
VP0432- footprint-F-6FAM	CGGAGCTTCGCTCTATTGTTGA	This study
VP0432- footprint-R	CAGAACCTAGCCCTTGTGTCTGA	This study
VP0432- footprint-F	CGGAGCTTCGCTCTATTGTTGA	This study
VP0432- footprint-R-6FAM	CAGAACCTAGCCCTTGTGTCTGA	This study
VPA2032- footprint-F-6FAM	TGATGATCTTGTGTTGCTGCG	This study
VPA2032- footprint-R	CCAACTTTTTTCGCCAGCAACAT	This study
VPA2032- footprint-F	TGATGATCTTGTGTTGCTGCG	This study
VPA2032- footprint-R-6FAM	CCAACTTTTTTCGCCAGCAACAT	This study
VPA1467- footprint-F-6FAM	ACAAAAACGTATCGGTGGGTGA	This study
VPA1467- footprint-R	TGCGCCAGCAGATCTTGTGT	This study
VPA1467- footprint-F	ACAAAAACGTATCGGTGGGTGA	This study
VPA1467- footprint-R-6FAM	TGCGCCAGCAGATCTTGTGT	This study
<i>EMSA</i>		
VP0907-EMSA-F1	AGTACATCATGATCGTCGTG	This study
VP0907-EMSA-R1	GCATTCATTTTGCTTGTTAC	This study
VP0907-EMSA-F2	GAATTTAAAGTGATATTTTG	This study
VP0907-EMSA-R2	TACAGAAATGAAAGGATAAG	This study
VPA1649-EMSA-F1	ACTGGCTAATGAGTGCTCTA	This study

VPA1649-EMSA-R1	GGTTTATCCTATAAAATTGCG	This study
VPA1649-EMSA-F2	CGTCACTCTAAAGTGCCAAAC	This study
VPA1649-EMSA-R2	GGCAAATTGGAAAGAAGTGTC	This study
VPA0755-EMSA-F1	CAGTCAGTAAAGCGGCACC	This study
VPA0755-EMSA-R1	GTTTGTGTGACTCATATCGCTTAC	This study
VPA0449-EMSA-F1	GTTAGCTCCCGCCCATTTCCT	This study
VPA0449-EMSA-R1	TACGGCTCAGCCCCCATCAA	This study
VP0432-EMSA-F1	CGATTGATTCATCACTCTTGGTG	This study
VP0432-EMSA-R1	GCGCCAAAATGGCATAGAAAG	This study
VPA2032-EMSA-F1	CAGTGTTAGCCGTTTCATTACGT	This study
VPA2032-EMSA-R1	CTAATCTGTTAACCAAAAACCTGGTG	This study
VPA1467-EMSA-F1	CACTTAGCCAACCCTTGTCTTT	This study
VPA1467-EMSA-R1	AACCAGTGTAACACAATCAACATC	This study
VPA1467-EMSA-F2	CGTTTTTTCAAGCAGGTTAGA	This study
VPA1467-EMSA-R2	CATTATAGGATTAATGGAATCCG	This study

^aUnderline: sequence of restriction enzyme sites, Bold: FLAG-tag sequence

Figure S1

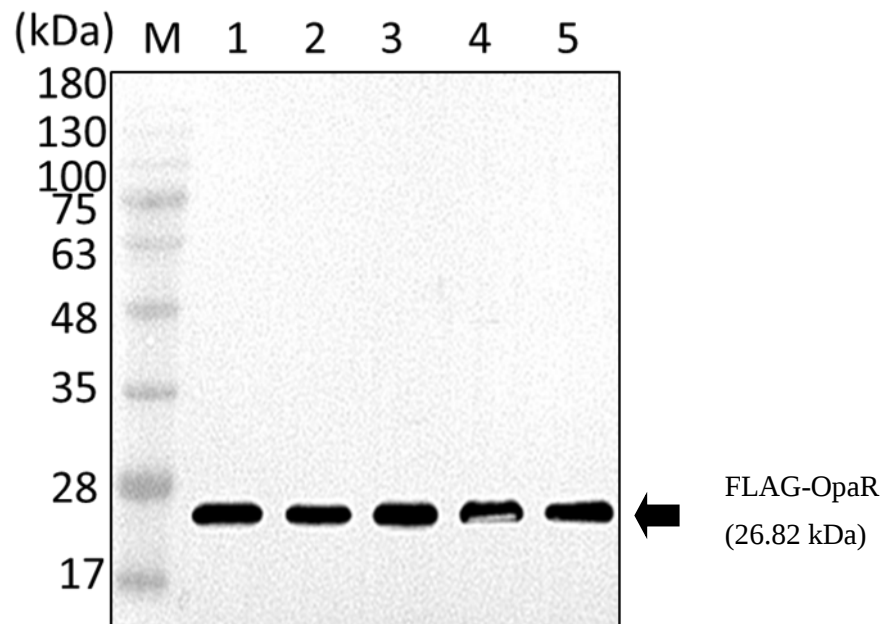


Figure S1 Expression levels of FLAG-OpaR at different growth phases.

Whole cell extract of FLAG-OpaR/VP93 was collected. Expression level of FLAG-OpaR was detected using Western blotting with anti-FLAG antibody in the early-log (lane 1: OD₆₀₀ 0.2), log (lane 2: OD₆₀₀ 0.5), late-log (lane 3: OD₆₀₀ 2.8), stationary (lane 4: OD₆₀₀ 4), and late-stationary (lane 5: OD₆₀₀ 5.2) phases. Marker, Prestained Protein Ladder 1025G (Biomann).