Supplementary Material

The cAMP Receptor Protein (CRP) of *Vibrio mimicus* Regulates its Bacterial Growth, Type II secretion system, Flagellum Formation, Adhesion Genes and Virulence

Ziqi Tian [1,†], Fei Xiang [1,2,†], Kun Peng [1], Zhenyang Qin [1], Yang Feng [1], Bowen Huang [1], Ping Ouyang [1], Xiaoli Huang [3], Defang Chen [3], Weimin Lai [1], Yi Geng [1,*]

[1] College of Veterinary Medicine, Sichuan Agricultural University, Chengdu 611130, China

[2] Agricultural and Rural Bureau of Zhongjiang County, Deyang 618100, China

[3] Department of Aquaculture, Sichuan Agricultural University, Chengdu 611130, China

† These authors contributed to this work equally.

*** Correspondence:**

Corresponding Author: Yi Geng

Address: Huimin Road No. 211, Chengdu, Sichuan Province, Chengdu 611130, China

Tel.: +86028-86291162

E-mail: gengyisicau@126.com

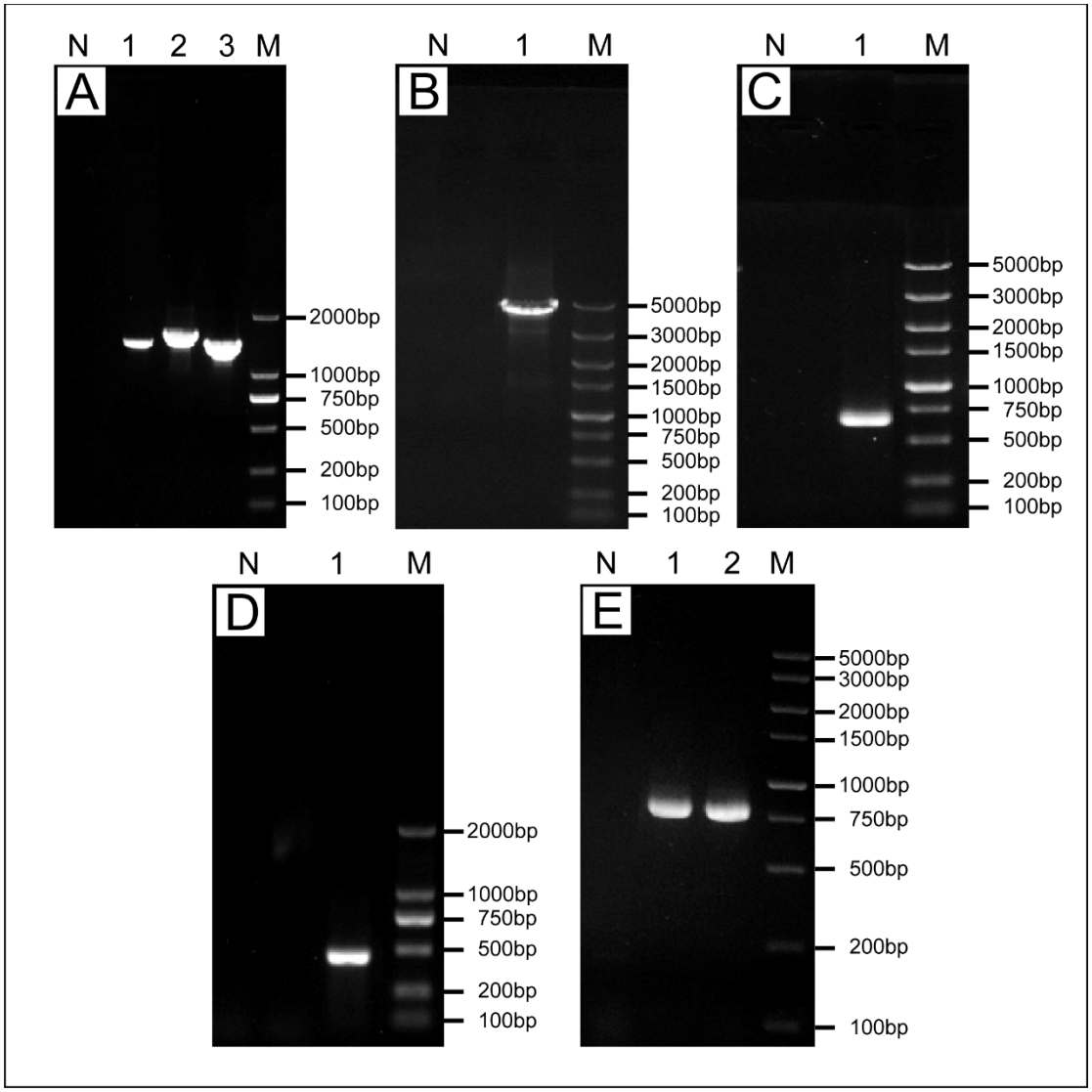

Fig S1. Construction and verification of the deletion and the complementation strains. A: Homologous recombination fragments amplified by primer pairs Up-F/R, Down-F/R and Kan^r-F/R, respectively (1: Upstream homology arm fragment, 2: *Kan*^{r} gene fragment, 3: Downstream homology arm fragment); B: Fusion fragment; C: Primer pair pKD46-F/R for identification of SCCF01/pKD46; D: Primer pair pCP20-F/R for identification of pCP20 plasmid; E: Primer pair pBAD24-F/R for identification of complementing strain; N: negative control; M: DNA Marker.

Table S1. Construction and detection Primers used in this study.

Primer	Primer sequences(forward/reverse,5′-3′)	Purpose
Up	F: ATCCTCAATGCACAATCCCAT R: CGAAGCAGCTCCAGCCTACACTTGCTTGTATCACACGGCAAC	Amplification of the 1378 bp DNA fragment upstream of the 633 bp target deletion region
Down	F: ATTCATATGGACCATGGCTAAGCTATCCATATTTTAGCGAAGCC R: ACTCGACAATTTGATCCGTGA	Amplification of the 1347 bp DNA fragment downstream of the 633 bp target deletion region
Kan^r	F: GTTGCCGTGTGATACAAGCAAGTGTAGGCTGGAGCTGCTTCG R: GGCTTCGCTAAAATATGGATAGCTTAGCCATGGTCCATATGAAT	Creation of the 1489 bp deletion allele
C-*crp*	F: GCGGAATTCATGGTTCTAGGTAAACCTCAA R: CCCAAGCTTTTAGCGAGTACCGTAAACCAC	Amplification of 651 bp *crp* coding fragment
Crp	F: ACGTTATCCAATGGCGAATG R: TACCGTGATCATGTGCACAG	Amplicons of 381 and 934 bp for identification of Δ*crp* and WT strains
Crp-RT-PCR	F: TAAACCTCAAACCGATCCAAC R: TTCAAACAAGCCGAGTTCACC	Amplification of 223 bp for identification of *crp* gene intragenic region
pKD46	F: ATGACACCGGACATTATCCTG R: TTTCAGCCAGTGCCTCGTC	Amplicons of 649 bp for identification of pKD46 plasmid recombinant strain
pCP20	F: TGCTCCACCCACAGGATGCT R: ACACTGGCACCGCCGTTACT	Amplicons of 474 bp for identification of pCP20 plasmid recombinant strain
pBAD24	F: ATGCCATAGCATTTTTATCC R: GATTTAATCTGTATCAGG	Amplicons of 798 bp for identification of complementing strain

Table S2. RT-qPCR Primers used in this study.

Genes	Primer sequences (5′-3′)	Size (bp)
16S	F: CGTGCTACAATGGCGTATACAGAGG R: GCGATTACTAGCGATTCCGACTTCA	125
vmh	F: CCTGAGTTATTGGTTGGTGGTACGG R: CGCCAGAGGTCGCTGTGATAGA	85
tlh	F: CCACGGCAGCAACTGAACCAT R: GGACGATACCAACAGCGGACATAAG	109
epsM	F: TGCTTATCTGCAAGAGCGACAAGG R: TGACTTCCACCACGCCATTTACTTT	83
epsL	F: ACCTGCTTGCTGCTTCCGATTG R: CGCCACACTTGCTGTTCCACTAA	106
epsI	F: TCGTGAGGGTAGTGAGCAGATGG R: TTTGAGCAGATTGTCGGCGGTTT	82
epsG	F: TGGCGGTTACATCAAGCGTCTG R: CCATCAGCACCTAGAGTGAACACAT	108
epsF	F: CAAGTATTGGCGGCGGCAGAA R: CACTCTGTTCACCACTGGCAATCA	118
epsE	F: TTCCTCTTCACTGCTTGGCGTATTG R: TGCGTTGCTCTTTGTCTGCTTCA	95
epsC	F: GGACCTGACCGATCCGAATGTAATG R: ATCATGTTGTTGACCATCACGCTCA	100
epsD	F: TCAATGCGGTGTCTAACGATTCCAA R: TGCGGTTGAGCCAGTGATAACAG	125
vpsR	F: GTACATGGTCGCTCGTGACACTC R: TGATGCGTTGCTGTTGGTATTGGA	88
vpsT	F: GCTCACACGAAAGTTAGCCCAAGA R: CGATGCACCACTGCCGAGTAAC	133
rbmA	F: GGTTACATTCAACATGCGGCCAGTA R: GCTTTCAGTCAAAGGTCCAGAAGGT	115
rbmA	F: GGCGTATTGAGATGACCTCCTGTG R: TGTGCGTTCTGGTTATGCGATTGA	84
mshA	F: GACTCACTACCAACCGCAGAGC R: GCAGATACATCAAGTACACCGCCAT	137
flaA	F: CAGCCGCTCGTGATCTGAAGTT R: ACCGTTGATGTAAGTCGCCAATTCT	116
flrA	F: CTTCACGCCACGAGCAATCAATTC R: TACAGGATGACCATACGCTCAACCA	96
flrC	F: GACTGCCTACGCCAACATTCAAGA R: ACCACCGCATCACCATTGTCATC	150

fliA	F: CGGATGCTGAAGTGGCGAAGTT R: ACGGTGAAATTGCGTCATCAGAAAC	129
fliD	F: TGAGTCCTGAAGAACGAGCCTACAT R: GACGCCATCCAGCACAACCAT	143
flrB	F: GCGGATCAGTGATTTACAGCGTCTT R: GCAGCAAGATTGGCGGCATACA	114
flgE	F: GCACAGCAGTTCCACGAAGGTT R: CGCCGTTACGAGTCAGCTCATT	133
flhA	F: GGCACAGTTGATTGGTCACGAAGA R: CAAGCGATAGTTGGTCAGGCACAA	104
flaB	F: CCGTGATACTGACTATGCTCGTGAA R: AGATGGTGACTGCTTCGCTTGC	103
acfD	F: CCTGAATACGGCACCACTGAATAGC R: ACCTCGGTAGCACCATCAACATTG	96
ompU	F: TCATCCGTGAGGCTGTCATTCAAG R: TGGTCGCTTCAGTACCTGAGTTCA	150
hapA	F: GTGTGAACGCCGCTTGTGGAA R: CGCCGCTACCGTGAATGTATAGAA	123

Table S3. Biochemical test results.

Biochemical test	**SCCF01**	**Δ*crp***
Ala-Phe-Pro-ARYLAMIDASE (APPA)	—	—
ADONITOL (ADO)	—	—
L-Pyrrolydonyl-ARYLAMIDASE (PyrA)	+	+
L-ARABITOL (IARL)	—	—
D-CELLOBIOSE (Dcel)	—	—
BETA-GALACTOSIDASE (BGAL)	+	+
H_2S PRODUCTION (H_2S)	—	—
BETA-N-ACETYL-GLUCOSAMINIDASE (BNAG)	+	+
Glutamyl Arylamidase pNA (AGLTp)	—	—
D-GLUCOSE (dGLU)	+	+
GAMMA-GLUTAMYL-TRANSFERASE (GGT)	—	—
FERMENTATION/ GLUCOSE (OFF)	—	—
BETA-GLUCOSIDASE (BGLU)	—	—
D-MALTOSE (dMAL)	+	—
D-MANNITOL (dMAN)	+	—
D-MANNOSE (dMNE)	—	—
BETA-XYLOSIDASE (BXYL)	—	—
BETA-Alanine arylamidase pNA (BAlap)	—	—
L-Proline ARYLAMIDASE (ProA)	—	—
LIPASE (LIP)	—	—

PALATINOSE (PLE)	—	—
Tyrosine ARYLAMIDASE (TyrA)	+	—
UREASE (URE)	+	+
D-SORBITOL (dSOR)	—	—
SACCHAROSE/SUCROSE (SAC)	—	—
D-TAGATOSE (dTAG)	—	—
D-TREHALOSE (dTRE)	+	—
CITRATE(SODIUM) (CIT)	—	—
MALONATE (MNT)	—	—
5-KETO-D-GLUCONATE (5KG)	—	—
L-LACTATE alkalinisation (ILATk)	+	—
ALPHA-GLUCOSIDASE (AGLU)	—	—
SUCCINATE alkalinisation (SUCT)	+	—
Beta-N-ACETYL-GALACTOSAMINIDASE (NAGA)	+	—
ALPHA-GALACTOSIDASE (AGAL)	—	—
PHOSPHATASE (PHOS)	+	—
Glycine ARYLAMIDASE (GlyA)	—	+
ORNITHINE DECARBOXYLASE (ODC)	+	—
LYSINE DECARBOXYLASE (LDC)	+	—
L-HISTIDINE assimilation (IHISa)	—	—
COURMARATE (CMT)	—	+
BETA-GLUCORONIDASE (BGUR)	—	—
O/129 RESISTANCE (O129R)	—	—
Glu-Gly-Arg-ARYLAMIDASE (GGAA)	+	—
L-MALATE assimilation (IMLTa)	—	—
ELLMAN (ELLM)	+	+
L-LACTATE assimilation (ILATa)	—	—