Supplement S1.

Plasmid constructions.

Two synthetic oligonucleotides Dksw-F and Dksw-R (Table S1) were designed from the available whole genome sequence information of *V. cholerae* (21) and these primers were used to PCR amplify the entire *dksA* gene (VC0596) along with its flanking regions using the genomic DNA of the strain N16961. The expected 841-bp PCR amplicon was gel purified followed by cloning in the PCR cloning vector pDrive (Table 1) and the recombinant plasmid thus constructed was designated pDDKW1 (Table 1). To construct the recombinant suicide plasmid pKDK1 (Table 1), initially a 297-bp junctional DNA fragment containing 188-bp of the 3'-end of the *dksA* gene along with its flanking sequences and 109-bp of the vector sequences including M13 reverse sequence was PCR amplified using the plasmid DNA pDDKW1 as a template and the primers Dksdel-F and M13-R (standard M13 reverse primer; Table S1) followed by double digestion of the DNA fragment with the enzymes *Bgl*II and *Pst*I, ligation of the digested product to similarly digested pDDKW1 and transformation of the ligation products in *E. coli* DH5α strain (Table 1). The recombinant clone carrying the Δ*dksA* allele was further double digested with the enzymes *Pst*I and *Hin*dIII and the insert DNA was ligated to similarly digested cloning vector pBluescript II KS+ (Table 1) to generate the plasmid pBSDA3.5 (Table 1). To generate the Δ*dksA*::*kan* allele, 1.2-kb kanamycin resistance gene (*kan*) cassette from the plasmid pUC4K (Amersham, UK; Table 1) was obtained by digestion with the enzyme *Bam*HI and cloned in the unique *Bgl*II site (*Bam*HI compatible) present in the insert DNA of the plasmid pBSDA3.5 and thus generating the recombinant plasmid pBSDA4.8 (Table 1). The plasmid pBSDA4.8 was double digested with *Kpn*I and *Sac*I to get 1.8-kb insert DNA containing the Δ*dksA*::*kan* allele followed by ligation with the similarly digested *R6K ori* containing suicide vector pKAS32

(Table 1), transformation of the ligation mixture into the *E. coli* strain S17-1λ*pir* (Table 1) and the recombinant plasmid thus obtained was called pKDK1 (Table 1). To construct *dksA* expression plasmid pDksA$_{BAD}$ (Table 1), the entire *V. cholerae dksA* ORF plus 39-bp of the downstream sequence (total size 496-bp) was PCR amplified using the primers DksorfF and DksorfR (Table S1) and the amplified DNA fragment was double digested with *Eco*RI and *Pst*I followed by cloning of the fragment into similarly digested expression vector pBAD24 (Table 1). Another *dksA* expression plasmid pDksA$_{Vc}$ (Table 1) was constructed by double digesting the plasmid pDDKW1 (Table 1) with the enzymes *Bam*HI and *Sal*I to get the 0.841-kb insert DNA containing the entire *dksA* gene with its natural promoter and was ligated with the similarly digested vector pBR322 (Table 1). To construct the *fliA* expression plasmid pFliA$_{BAD}$ (Table 1), the entire *fliA* ORF of 780-bp of *V. cholerae* was PCR amplified using the primers FliAorf-F and FliAorf-R (Table S1) and the genomic DNA of *V. cholerae* strain N16961 as templates. The amplified DNA fragment was double digested with the enzymes *Kpn*I and *Pst*I followed by cloning of the fragment into similarly digested expression plasmid pBAD24 (Table 1). To construct the *relV* expression plasmid pRelV$_{BAD}$ (Table 1), the entire *relV* ORF of *V. cholerae* with 20-bp of the downstream sequence (total size 812-bp) was PCR amplified using the primers VCRVorf-F and VCRVorf-R (Table S1) and the genomic DNA of *V. cholerae* strain N16961 as templates. The amplified DNA fragment was double digested with the enzymes *Eco*RI and *Hin*dIII followed by cloning of the fragment into similarly digested expression plasmid pBAD24 (Table 1). The authenticity of all the plasmid constructs was confirmed by restriction enzyme digestion as well as by DNA sequencing (data not shown).

Construction of strains.

The recombinant suicide plasmid pKDK1 (Table 1) carrying the Δ*dksA*::*kan* allele was utilized to delete the chromosomal *dksA* gene of *V. cholerae* O1 El Tor strains N16961 and C6709 (Table 1) essentially as described previously (13). Briefly, the suicide plasmid pKDK1 (Amp^r Kan^r) was conjugally transferred from *E. coli* S17-1λ*pir* into *V. cholerae* cells and ampicillin and kanamycin resistant transconjugants were selected as chromosomal first cross-over event followed by selection of ampicillin sensitive and kanamycin resistant cells by double cross over event and thus generating the desired in frame *dksA* deletion mutant of *V. cholerae*. The Δ*dksA* mutants developed from wild-type N16961 and C6709 strains were designated N-DksA1 and C-DksA1 (Table 1), respectively, and confirmed by a PCR assay using the primers Dksfl-F/Dksfl-R (Table S1). Similarly, deletion of the *relV* gene (14) in the genome of the Δ*dksA* strain N-DksA1 was done using the previously reported suicide plasmid pBS20 (Table 1) and the Δ*dksA* Δ*relV* double mutant thus constructed was designated NRVDK2 (Table 1). Presence of right allele in right locus in the genomes of mutant *V. cholerae* strains was confirmed by PCR, reverse transcriptase-PCR (RT-PCR), Southern hybridization, and by DNA sequencing (data not shown).

Supplemental Table S1. Oligonucleotides used in this study.

Oligonucleotides	Sequence (5'→3')	Reference
Dksint-F	5'- TATGTCACCTGGGCAAATCA-3'	This study
Dksrt-R	5'- TCAGCCAGTGTTTTGCAAT -3'	This study
Dksw-F	5'- CAAGAAAAGGGAGCTTGTGC-3'	This study
Dksw-R	5'- CGGAAGTCGCTAAAGATGGA-3'	This study
Dksdel-F	5'-GAAGATCTAAGAGGAGG-3' *Bgl*II	This study
DksorfF	5'-GGAATTCTGTATGACAGAGTC-3' *Eco*RI	This study
DksorfR	5'-AAAACTGCAGCTTGTGCTCC-3' *Pst*I	This study
Dksfl-F	5'-AGTTGGGAACTGACGCAGAC-3'	This study
Dksfl-R	5'-ACCAGCCAAGTTCCTTGTTG-3'	This study
recA-F	5'-GCAATTTGGTAAAGGCTCCA-3'	This study
recA-R	5'-GTTGTGCAGCAGCAATCAGT-3'	This study
FliA-F	5'-CGCGAGCTTTGAAACCTATG-3'	This study
FliA-R	5'-ATCTTCCCCCTCTTCAAACG-3'	This study
FliAorf-F	5'- GGGGTACCTTCGGTGAATAAAG-3' *Kpn*I	This study
FliAorf-R	5'-AACTGCAGCCACTGAGTTTGAG-3' *Pst*I	This study
M13-R	5'-AACAGCTATGACCATG-3'	New England Biolabs
VC0595-F	5'-CAACAAGGAACTTGGCTGGT-3'	This study
VC0595-R	5'-GCCCTGTCGGTTCTATCAAA-3'	This study
PcnB-F	5'-ACGCGTTATCGTGAAGATCC-3'	This study
PcnB-R	5'-CCAAATCCAACATCTGCTCA-3'	This study
HapA-F	5'-TCCGGTGGTATTAACGAAGC-3'	This study
HapA-R	5'-ACGGCAAACACTTCAAAACC-3'	This study
VCRVorf-F	5'-CCGGAATTCTGAATGAGTCTATTGC-3' *Eco*RI	This study
VCRVorf-R	5'- CCCAAGCTTGGGTCGGGTATTTATG-3' *Hin*dIII	This study