

Table 1. Bacterial strains and plasmids used in this study

Strains or plasmids	Descriptions	Reference or source	
Strains			
<i>K. pneumoniae</i>			
CG43S3	CG43 Sm ^r	(52)	
CG43S3-Ah01	CG43S3 $\Delta kvhA$	This study	
CG43S3-Z01	CG43S3 $\Delta lacZ$	This study	
CG43S3-S01	CG43S3-Z01 $\Delta kvgS$	This study	
CG43S3-Sh01	CG43S3-Z01 $\Delta kvhS$	This study	
CG43S3-AZ18	CG43S3-Z01 $\Delta kvgA$	This study	
CG43S3-RZ01	CG43S3-Z01 $\Delta kvhR$	This study	
CG43S3-AhZ01	CG43S3-Z01 $\Delta kvhA$	This study	
CG43S3-AAh01	CG43S3-Z01 $\Delta kvgA \Delta kvhA$	This study	
CG43S3-AAR01	CG43S3-Z01 $\Delta kvgA \Delta kvhR$	This study	
CG43S3-AhR01	CG43S3-Z01 $\Delta kvhA \Delta kvhR$	This study	
CG43S3-AAhR01	CG43S3-Z01 $\Delta kvgA \Delta kvhA \Delta kvhR$	This study	
CG43S3-RcsBZ01	CG43S3-Z01 $\Delta rcsB$	This study	
CG43S3-RpoS01	CG43S3-Z01 $\Delta rpoS$	This study	
U9451	CG43S3 $\Delta galU$	(52)	
U9451- <i>kvgA</i> ⁻	U9:	This study	
U9451- <i>kvhA</i> ⁻	U9:	This study	
U9451- <i>kvhR</i> ⁻	U9:	This study	
<i>E. coli</i>			
JM109	<i>rec.</i> (<i>lac</i>	96 <i>relA1 thi</i> <i>traD36]</i>	Laboratory stock
BL21-CodonPlus(DE3)-RIL	<i>F. o.</i> <i>ileY</i>	<i>idA Hte [argU</i>	Laboratory stock
S17-1 λ <i>pir</i>	<i>hsdK recA pro RP4-2 [1c::Mu; Km::Tn7] (λpir)</i>	(84)	
Plasmids			
pKAS46	Positive selection suicide vector, <i>rpsL</i> Ap ^r Km ^r	(84)	
pET30a-c	His-tagging protein expression vector, Km ^r	Novagen	
pUC-T	TA cloning vector	MDBio	
pYC016	Promoter selection vector, LuxAB ⁺ Cm ^r	(52)	
placZ15	A derivative of pYC016, containing a promoterless <i>lacZ</i> from <i>K. pneumoniae</i> CG43S3 as the reporter, Cm ^r	This study	
placZ16	2-kb fragment containing an internal 1.5-kb deletion in <i>lacZ</i> cloned into pKAS46, Ap ^r Km ^r	This study	
pETm-C	A derivative of pET30C, containing malonate promoter, Km ^r	This study	
pA13	2-kb fragment containing an internal 0.6-kb deletion in <i>kvgA</i> cloned into pKAS46, Ap ^r Km ^r	This study	
pAhm1	2-kb fragment containing an internal 0.7-kb deletion in <i>kvhA</i> cloned into pKAS46, Ap ^r Km ^r	This study	
pR14	pKAS46 carrying a $\Delta kvhR$ fragment	This study	
pYC220	2.0-kb fragment containing a 763-bp deletion in <i>rscB</i> locus cloned into pKAS46	(53)	

pRcsB2	The DNA fragment carrying entire <i>rcsB</i> coding sequence cloned into the <i>EcoRV/SalI</i> site of pETm-c	This study
pYC030	1.9-kb fragment, containing <i>kvgS</i> with 1.0-kb of an internal <i>BamHI</i> deletion, cloned into pKAS46	(51)
pSh01	2.3-kb fragment, containing <i>kvhS</i> with 1.0-kb of an internal <i>EcoR</i> deletion, cloned into pKAS46	This study
pRpoS09	1.6-kb fragment, containing <i>rpoS</i> with Tc gene in <i>BamHI</i> site, cloned into pKAS46	This study
pkvgA1	A fragment of <i>K. pneumoniae</i> CG43S3 <i>kvgA</i> gene generated by PCR, and cloned into pUC-T, Ap ^r	This study
pkvgA4	Deletion of the receiver domain of <i>kvgA</i> gene digested by <i>ClaI</i> , and cloned into pET30c, Km ^r	This study
pHP4004	A fragment of <i>K. pneumoniae</i> CG43S3 <i>kvhA</i> gene generated by PCR, and cloned into pUC-T, Ap ^r	This study
pHP4005	A <i>kvhA</i> gene containing DNA from <i>K. pneumoniae</i> CG43S3 digested with <i>BamHI</i> and cloned into pET30c, Km ^r	This study
pR28	A fragment of <i>K. pneumoniae</i> CG43S3 <i>kvhR</i> gene generated by PCR, and cloned into pUC-T, Ap ^r	This study
pR31	Deletion of the receiver domain of <i>kvhR</i> gene digested by <i>EcoRV/HindIII</i> , and cloned into pET30a, Km ^r	This study
pA16	399-bp <i>BamHI</i> fragment containing the putative <i>kvgAS</i> promoter, cloned into <i>BamHI</i> site of placZ15	This study
pA23	360-bp <i>BamHI/BglII</i> fragment containing the putative <i>BamHI</i> site of placZ15	This study
pA26	318-bp <i>BamHI/BglII</i> fragment containing the putative <i>kvg</i> site of placZ15	This study
pA28	196-bp <i>BamHI/BglII</i> fragment containing the putative <i>kvg</i> site of placZ15	This study
pA30	52-bp <i>BamHI/BglII</i> fragment containing the putative <i>BamHI</i> site of placZ15	This study
pAh01	516-bp <i>BamHI/BglII</i> fragment containing the putative <i>kvhAS</i> promoter, cloned into <i>BamHI</i> site of placZ15	This study
pAh02	374-bp <i>BamHI/BglII</i> fragment containing the putative <i>kvhAS</i> promoter, cloned into <i>BamHI</i> site of placZ15	This study
pAh03	180-bp <i>BamHI/BglII</i> fragment containing the putative <i>kvhAS</i> promoter, cloned into <i>BamHI</i> site of placZ15	This study
pRP05	500-bp <i>BamHI</i> fragment containing the putative <i>kvhR</i> promoter, cloned into placZ15	This study
pA415	A 1.3 kb <i>EcoRI</i> fragment containing <i>kvhA</i> locus with the putative promoter cloned into pRK415	This study
pAHm	A <i>BamHI</i> fragment of pHP4005 carrying entire <i>kvhA</i> coding sequence cloned into the <i>BamHI</i> site of pETm-c	This study
pRC01	A 1.2 kb <i>BamHI/EcoRI</i> fragment containing <i>kvhR</i> locus with the putative promoter cloned into pACYC184	This study
pRC02	A 1.2 kb <i>BamHI/EcoRI</i> fragment containing <i>kvhR</i> locus with the putative promoter cloned into pRK415	This study
pOrf12	500-bp <i>BamHI</i> fragment containing the putative <i>orf1-2</i> promoter, cloned into placZ15	This study
pOrf315	900-bp <i>BamHI</i> fragment containing the putative <i>orf3-15</i> promoter, cloned into placZ15	This study



pOrf1617	300-bp <i>Bam</i> HI fragment containing the putative <i>orf16-17</i> promoter, cloned into <i>placZ15</i>	This study
pKatG02	430-bp <i>Bam</i> HI/ <i>Bgl</i> II fragment containing the putative <i>katG</i> promoter, cloned into <i>Bam</i> HI site of <i>placZ15</i>	This study
pKatE02	815-bp <i>Bam</i> HI/ <i>Bgl</i> II fragment containing the putative <i>katG</i> promoter, cloned into <i>Bam</i> HI site of <i>placZ15</i>	This study
pSodC02	160-bp <i>Bam</i> HI/ <i>Bgl</i> II fragment containing the putative <i>sodC</i> promoter, cloned into <i>Bam</i> HI site of <i>placZ15</i>	This study



Table 2. Primers used in this study

Primer no.	Sequence	Complementary position
lac01	5'-GCGAACGACAAGATCTGACTTA-3'	-24 relative to the <i>lacZ</i> start codon
lac02	5'-ATTATGCCGTTCTAGAGGCG-3'	+103 relative to the <i>lacZ</i> stop codon
lac03	5'-TGAAACGCAAGGATCCGAGC-3'	+1444 of the <i>lacZ</i> coding region
lac05	5'-CAGGTGGAGGAGCTCGAAAG-3'	-907 relative to the <i>lacZ</i> start codon
lac06	5'-AAACGGGATCCGCTGGCA-3'	+117 of the <i>lacZ</i> coding region
lac07	5'-GCAGTGCCTCTAGATCGT-3'	+2498 of the <i>lacZ</i> coding region
a02	5'-CAATATCATAGCCAGCA-3'	+45 relative to the <i>kvgA</i> stop codon
a03	5'-ATTGCTTCACTCACCT-3'	-32 relative to the <i>kvgA</i> start codon
a08	5'-GAGAGCTCGATTATTCATCGA-3'	-834 relative to the <i>kvgA</i> start codon
a09	5'-CATATTGTGGATCCTGCTGTT-3'	+22 of the <i>kvgA</i> coding region
a10	5'-CGATGCGGGATCCAATGCCTTTA-3'	+296 of the <i>kvgA</i> coding region
a11	5'-AACAAGATCTAGCTTTTGAT-3'	+699 relative to the <i>kvgA</i> stop codon
a14	5'-ATTTTCAGGATCCACCACCTT-3'	-409 relative to the <i>kvgA</i> start codon
a15	5'-TGCCTGGATCCGTGATTAG-3'	-204 relative to the <i>kvgA</i> start codon
a17	5'-GGTAACTAACGGATCCACTC-3'	-320 relative to the <i>kvgA</i> start codon
a18	5'-AGATCTGTTCTGAATTTATTC-3'	-361 relative to the <i>kvgA</i> start codon
a19	5'-AGATCTGGTGGTACCACGATAC-3'	-52 relative to the <i>kvgA</i> start codon
AS02	5'-CAGCCATGCTTTCTCCTT-3'	+156 relative to the <i>kvhA</i> stop codon
AS03	5'-TCTTATTTATCCGTCG-3'	+1 relative to the <i>kvhS</i> start codon
AS04	5'-ATCTGCAGAATATCCC	532 of the <i>kvhS</i> coding region
AS07	5'-ATCAGGATCCACGCC	8 relative to the <i>kvhA</i> start codon
AS08	5'-GACTTATCGGCAATAT	942 of the <i>kvhS</i> coding region
AS09	5'-GGAAAAAACTGACAA	+2 relative to the <i>kvhS</i> stop codon
AS12	5'-TCCTGCAATGCTGGA	245 relative to the <i>kvhA</i> start codon
AS16	5'-GCCCCGGTTATTTTTA	2 relative to the <i>kvhA</i> start codon
AS23	5'-CATGGCGGTTTCGTCTT	relative to the <i>kvhS</i> start codon
A201	5'-GTGAAAAAGCTTCGT	16 relative to the <i>kvhA</i> start codon
A203	5'-CAACGACAGCTCTTCC	+9 of the <i>kvhA</i> coding region
R01	5'-CTTTTAAAGCTTAAATGA-3'	-469 relative to the <i>kvhR</i> start codon
R02	5'-TTCGGGTACCTCTCCATC-3'	+62 relative to the <i>kvhR</i> start codon
R04	5'-AGGCCTTCAATCCCACAC-3'	+23 relative to the <i>kvhR</i> stop codon
R07	5'-AGGTTAAGAGCTCCAGCGCC-3'	-1097 relative to the <i>kvhR</i> start codon
R09	5'-TGGATCCGTTTGTATGAATGA-3'	+353 of the <i>kvhR</i> coding region
P074	5'-ACTGGATCCACGATCATGGATAAGAT-3'	-724 relative to the <i>orf1</i> start codon
P075	5'-ACTGGATCCTGCGACCGGAATAACC-3'	+42 of the <i>orf1</i> coding region
P040	5'-ACTGGATCCAGGCCTGGTAATAGCCATT-3'	-890 relative to the <i>orf3</i> start codon
P041	5'-ACTGGATCCCCTGTCGTATCTCAATG-3'	+60 of the <i>orf3</i> coding region
P045	5'-GGTGCAGATCTATAAGC-3'	-307 relative to the <i>orf16</i> start codon
P046	5'-ACTGGATCCAGACGGAGGAAGTGTTC-3'	+89 of the <i>orf16</i> coding region
AP01	5'-GCTGCTGAGATCTGCCGC-3'	+99 of the <i>kvhA</i> coding region
AP02	5'-GAACGCCGGATCCTACAGC-3'	-188 relative to the <i>kvhA</i> start codon
A201	5'-GGATCCGAAAAAGGATCGTTCA-3'	-516 relative to the <i>kvhA</i> start codon
A202	5'-GGATCCCCAGTACTGTTATTCC-3'	-374 relative to the <i>kvhA</i> start codon
rpoS01	5'-ACGATGATTACCTGAGTGCCT-3'	-291 relative to the <i>rpoS</i> start codon
rpoS02	5'-TTGAGCGGTGAGAAGATG-3'	+47 relative to the <i>rpoS</i> stop codon
rpoS04	5'-GGATCCCTGAGCAAAGCACC-3'	+33 of the <i>rpoS</i> coding region
rpoS05	5'-CTAGATCTCCTGGGTCACCG-3'	-869 relative to the <i>rpoS</i> start codon
K01	5'-CGGATCCATTGTTGGATG-3'	+36 of the <i>katG</i> coding region
K02	5'-CACGCTGATAGATCTGTATTC-3'	-422 relative to the <i>katG</i> start codon
E01	5'-CGGGTGCTTATCAGATCTTAC-3'	+18 of the <i>katE</i> coding region



E02	5'-CTGGATCCGATGTGGATTG-3'	-803 relative to the <i>katG</i> start codon
D01	5'-GCGAGGGATAAGATCTCG-3'	+34 of the <i>sodC</i> coding region
D02	5'-CAGCAGTGGATCCGCATC-3'	-121 relative to the <i>sodC</i> start codon



Table 3. Effect of *kvgA*, *kvhA*, and *kvhR* gene deletion on activities of P_{kvgAS} , P_{kvhAS} , and P_{kvhR}

Strains	β -galactosidase activity (Miller units)		
	Mean \pm SD (%Z01 ^a)		
	pA16 ($P_{kvgAS}::lacZ$)	pAh01 ($P_{kvhAS}::lacZ$)	pRP05 ($P_{kvhR}::lacZ$)
Z01	448 \pm 3 (100)	226 \pm 13 (100)	374 \pm 6 (100)
AZ18	232 \pm 4 (52)	166 \pm 8 (74)	250 \pm 8 (67)
AhZ01	431 \pm 2 (96)	228 \pm 11 (100)	237 \pm 5 (63)
RZ01	438 \pm 9 (98)	232 \pm 6 (102)	174 \pm 10 (47)

^a Compared with Z01 carrying the testing reporter plasmid.



Table 4. Characterization of the *K. pneumoniae* CG43S3-Z01 derived mutants.

Strains	CPS amounts		LD ₅₀ (CFU×10 ³)
	(Mean quantity ± SD ^a)	% Z01	
Z01	22.8 ± 3.8	100	3
RcsBZ01	11.6 ± 2.8	51	ND ^b
AZ18	15.7 ± 0.3	68	275
AhZ01	24.4 ± 2.4	107	3
RZ01	13.6 ± 1.5	59	300
AAh01	11.9 ± 2.2	52	400
AR01	12.9 ± 0.8	56	400
AhR01	17.6 ± 0.9	77	300
AAhR01	15.1 ± 0.3	66	400

^a Values are the averages of triplicate samples and are given as micrograms of uronic acid per 10⁹ CFU.

^b ND, not determined.



Table 5. Effect of over-expression of *kvhA* to drug susceptibility in *K. pneumoniae* CG43S3

Antibiotics ($\mu\text{g/disk}$)	Inhibition zone (mm) ^a		
	CG43S3	CG43S3 [pHAM]	CG43S3[pHAM _{dHTH}]
Fosfomicin (50)	22	31	21
Cephalothin (30)	21	13	20
Piperacillin (100)	25	16	26
Carbenicillin (100)	14	7	14

^a Diameter of zones of inhibition, measured across disks of 6 mm diameter.



Table 6. Analysis of the target genes that are regulated by using a promoter trapping approach

Clones	Insert size (bp)	β -galactosidase activity ^a (mean \pm SD)			homolog	
		Wild type	<i>kvgA</i> mutant	Fold reduction	Gene (% identity)	Length (bp)
krg01	1222	58.8 \pm 1.3	203.7 \pm 13.1	3.4	<i>cps</i> gene cluster for ORF12 (100)	831
					oligogalacturonate lyase (64)	255
krg30	801	71.6 \pm 8.3	302.3 \pm 21.6	4.2	DNA primase (60)	468
					ATP-independent RNA helicase <i>dbpA</i> (81)	333
krg32	843	43.6 \pm 7.4	288.5 \pm 1		<i>murB</i> promoter region (100)	120
					RepA/RepC (100)	252
					SocE (50)	346
					hypothetical protein (100)	125
krg89	218	36.4 \pm 3.2	200.7 \pm 2		<i>lacZ</i> (89)	153



a The activity of the bacteria grown in LB medium to stationary-phase are shown in Miller units.

Table 7. Homology of the sequences obtained by cDNA subtractive hybridization analysis.

Clones	Insert length (bp)	Annotated number in KP NTUH-K2044	Homologous sequence (% identity)	Functions
KvhA activated genes				
hag2-1	1125	KP0224 235977~237317	DcuB (99)	anaerobic C4-dicarboxylate transporter
hag3-4	777	KP1104 1077926~1078942	CsiD (99)	RpoS dependent gene
hag4-1	559	KP3082 2935625~2936551	Transcriptional regulator (98)	AraC-type proteins DNA-binding protein
hag5-1	388	KPP161 110396~110551	Transcriptional regulator (100)	conserved hypothetical protein
KvhA repressed genes				
hrg4-1	439	KP0082 80778~81740		6-phosphofructokinase
hrg1-6	725	KP1100 1072324~107372		γ -aminobutyric acid (GABA) transporter
hrg2-6	571	KP4445 4267334~42684		Membrane lytic murein transglycosylase A



Table 8. The proteins identified by MALDI/TOF analysis

Spot's number	Protein name and/or descriptions	Differential expression in bacteria ^a			
		U9451	U9451- <i>kvgA</i> ⁻	U9451- <i>kvhA</i> ⁻	U9451- <i>kvhR</i> ⁻
001	Thioredoxin-dependent hydroperoxide peroxidase	+	-	-	-
002	Cytochrome c5	+	-	-	-
005	Hypothetical protein YtmB	-	±	+	±
007	Transcription elongation factor GreA		±	+	±
008	Orotidine 5'-phosphate decarboxylase (OMP de		±	+	±
009	PTS system, glucose-specific IIA component (Glucose-permease IIA component) (Phosphoenzyme II, A component)		±	+	±
010	Tellurium resistance protein <i>terE</i>		±	+	±
011	Tellurium resistance protein <i>terD</i>	-	±	+	±
015	50S ribosomal protein L10	-	±	+	±
016	50S ribosomal protein L9	-	±	+	±
021	Pyruvate formate lyase, <i>yfiD</i>	-	±	+	±



^a + means higher expression level; - means lower expression level. ± means no differential expression level.