



Supplementary Data and Additional Files

Novel Virulence Factors Deciphering *Klebsiella pneumoniae* KpC4 Infect Maize as a Crossing-Kingdom Pathogen: An Emerging Environmental Threat

Additional Table S1. Annotation of open reading frames (ORFs) of capsular polysaccharide synthesis (*cps*) loci in KpC4.

ORF no.	ORF name	ORF location	ORF homolog characteristics
1	<i>galF</i>	KpC4_1048	UTP-glucose-1-phosphate uridylyltransferase
2	<i>ORF2</i>	KpC4_1049	acid phosphatase
3	<i>wzi</i>	KpC4_1050	capsule assembly protein
4	<i>wza</i>	KpC4_1051	polysaccharide export protein
5	<i>wzb</i>	KpC4_1052	protein-tyrosine-phosphatase
6	<i>wzc</i>	KpC4_1053	tyrosine protein kinase
7	<i>wbaP</i>	KpC4_1054	Undecaprenyl-phosphate galactose phosphotransferase
8	<i>ORF7</i>	KpC4_1055	glycosyl transferase
9	<i>ORF8</i>	KpC4_1056	glycosyl transferase/amylovoran biosynthesis protein AmsE
10	<i>ORF9</i>	KpC4_1057	glycosyl transferase
11	<i>ORF10</i>	KpC4_1058	glycosyl transferase
12	<i>ORF11</i>	KpC4_1059	O-antigen and lipid-linked capsular repeat unit polymerase
13	<i>ORF12</i>	KpC4_1060	IS630 family transposase
14	<i>ORF13</i>	KpC4_1061	glycoside hydrolase
15	<i>ORF15</i>	KpC4_1062	O-antigen flippase
16	<i>gnd</i>	KpC4_1063	Gluconate-6-phosphate dehydrogenase
17	<i>ugd</i>	KpC4_1064	UDP-glucose 6-dehydrogenase 尿嘧啶雙磷酸葡萄糖去氫酶
I	<i>wzy</i>	KpC4_4152	common antigen polymerase
II	<i>wecF</i>	KpC4_4153	4-alpha-L-fucosyltransferase
III	<i>wzx</i>	KpC4_4154	O-antigen translocase

Additional Data from Table S2. Adhesins gene cluster of KpC4 and related *Klebsiella*.

Gene cluster	Description	CDS ^A	Found in				
			KpC4	K2044	78578	342	Kp13
matB fim	Fimbrillin matB homolog	KpC4_3300	+	+	+	+	+
	fimbrial protein fimK	KpC4_0254	+	+	+	+	+
	fimbrial protein fimH	KpC4_0255	+	+	+	+	+
	type 1 fimbrial protein fimG	KpC4_0256	+	+	+	+	+
	type 1 fimbrial protein fimF	KpC4_0257	+	+	+	+	+
	Outer membrane usher protein fimD	KpC4_0258	+	+	+	+	+
	Molecular chaperone FimC	KpC4_0259	+	+	+	+	+
	Fimbrin-like protein fimI	KpC4_0260	+	+	+	+	+
	type-1 fimbrial protein subunit A	KpC4_0261	+	+	+	+	+
	Type 1 fimbriae regulatory protein FimE	KpC4_0263	+	+	+	+	+
	Type 1 fimbriae regulatory protein fimB	KpC4_0264	+	+	+	+	+
	Type III fimbrial protein mrkA precursor	KpC4_0269	+	+	+	+	+
	Type III fimbriae chaperone MrkB	KpC4_0270	+	+	+	+	+
	Fimbrial protein MrkC	KpC4_0271	+	+	+	+	+
mrk	Fimbrial protein mrkD	KpC4_0272	+	+	+	+	+
	type III fimbriae protein mrkF	KpC4_0273	+	+	+	+	+

Additional data from Table S2. Continued.

Gene cluster	Description	CDS ^A	Found in				
			KpC4	K2044	78578	342	Kp13
<i>kpa</i>	Fimbrial protein	KpC4_3980	+	+	+	+	+
	Fimbrial assembly protein	KpC4_3979	+	+	+	+	+
	Fimbrial biogenesis outer membrane usher protein	KpC4_3978	+	+	+	+	+
	Fimbrial morphology protein	KpC4_3977	+	+	+	+	+
	Fimbrial protein	KpC4_3976	+	+	+	+	+
<i>kpb</i>	Putative transcriptional regulator protein	KpC4_3893		+	+	+	+
	Probable adhesin		—	+	+	+	+
	Outer membrane usher protein		—	+	+	+	+
	FimC-like chaperone protein		—	+	+	+	+
	Fimbrial protein		—	+	+	+	+
<i>kpc</i>	Recombinase involved in phase variation		—	+	—	—	+
	Fimbrial protein		—	+	—	—	+
	Putative fimbrial chaperone protein precursor		—	+	—	—	+
	Long polar fimbrial outer membrane usher		—	+	—	—	+
	putative fimbrial adhesin precursor		—	+	—	—	+
<i>kpd</i>	Fimbrial protein	KpC4_3086	+	+	+	+	+
	Outer membrane usher protein	KpC4_3087	+	+	+	+	+

Additional Data from Table S2. Continued.

Gene cluster	Description	CDS ^A	Found in				
			KpC4	K2044	78578	342	Kp13
	Fimbrial chaperone	KpC4_3088	+	+	+	+	+
	Fimbrial protein	KpC4_3089	+	+	+	+	+
	Putative transcriptional regulator	KpC4_3090					
<i>kpe</i>	Putative fimbrial-like protein	KpC4_1888	+	+	+	+	+
	Fimbrial protein/Chaperone protein	KpC4_1889	+	+	+	+	+
	Fimbrial protein	KpC4_1890	+	+	+	+	+
	Fimbrial protein	KpC4_1891	+	+	+	+	+
<i>kpf</i>	Fimbrial subunit type 1 precursor		—	+	+	± (58%)	+
	pilus assembly protein		—	+	+	± (60%)	+
	Outer membrane usher protein		—	+	+	—	+
	Putative fimbrial protein		—	+	+	—	+
<i>kpg</i>	Fimbrial protein	KpC4_0560	+	+	+	+	+

Gene cluster	Description	CDS ^Δ	Found in				
			KpC4	K2044	78578	342	Kp13
	Export and assembly usher protein of type I fimbriae	KpC4_0559	+	+	+	+	+
	fimbrial assembly protein	KpC4_0558	+	+	+	+	–
	Fimbrial protein	KpC4_0557	+	+	+	± (69%)	+

Δ, locus of the CDS in KpC4. +, gene found with BLASTP identity ≥ 90% and at least 80% coverage. –, gene absent according to the same criteria. ±, presence of a similar sequence, but which did not pass the constraints (% BLASTP similarity).

Additional Data from Table S3. Iron scavenging systems of KpC4 and related *Klebsiella*.

Category	System	Gene	Gene products	Role	CDS ^A	Find in				
						KpC4	78578	K2044	Kp13	342
Feo	Feo	<i>feoABC</i>	Fe(2+) transporter permease subunit FeoB	Fe ²⁺ transport	KpC4_4660	+	–	+	–	–
			ferrous iron transporter C		KpC4_4659	+	–	+	–	–
ABC transporter	Sit	<i>sitABCD</i>	metal ABC transporter permease	Fe ²⁺ transport	KpC4_0468	+	+	+	+	+
			metal ABC transporter permease		KpC4_0469	+	+	+	+	+
			manganese/iron transporter ATP-binding protein		KpC4_0470	+	+	+	+	+
			metal ABC transporter substrate-binding protein		KpC4_0471	+	+	+	+	+
	Kfu	<i>kfuA</i>	iron(III)-binding periplasmic protein	Fe ³⁺ transport	KpC4_2569	+	+	+	+	+
		<i>kfuB</i>	<i>iron(III)-transport system permease</i>		KpC4_2568	+	+	+	+	+
		<i>kfuC</i>	<i>iron(III)-transport ATP-binding protein</i>		KpC4_2567	+	+	+	+	+
	Fec	<i>fecB</i>	iron(III) dicitrate transport ATP-binding protein	Ferric citrate transport	KpC4_1411	+	+	+	+	+
		<i>fecD</i>	iron(III) dicitrate transport system permease		KpC4_1410	+	+	+	+	+
		<i>fecE</i>	iron(III) dicitrate transport ATP-binding protein		KpC4_1409	+	+	+	+	+
Hemophore based		<i>fpbABC</i>		Fe ³⁺ transport		–	–	+	–	+
	Hmu	<i>hmuRSTUV</i>	heme ABC transporter ATP-binding protein	Heme utilization	KpC4_0458	+	+	+	+	+

Additional Data from Table S3. Continued.

Category	System	Gene	Gene products	Role	CDS ^A	Find in				
						KpC4	78578	K2044	Kp13	342
Hemophore based	Hmu	<i>hmuRSTUV</i>	iron ABC transporter permease		KpC4_0459	+	+	+	+	+
			hemin ABC transporter substrate-binding protein		KpC4_0460	+	+	+	+	+
			hemin-degrading factor		KpC4_0461	+	+	+	+	+
			TonB-dependent receptor		KpC4_0462	+	+	+	+	+
		<i>fepA</i>	outer membrane receptor FepA		KpC4_4005	+	+	+	+	+
Siderophore based	Fep	<i>fepB</i>	ferric enterobactin (enterochelin) binding protein		KpC4_2955	+	+	+	+	+
		<i>fepC</i>	Ferric enterobactin transport ATP-binding protein		KpC4_2959	+	+	+	+	+
		<i>fepD</i>	ferric enterobactin transport system permease protein		KpC4_2957	+	+	+	+	+
		<i>fepG</i>	ferric enterobactin transport system permease protein		KpC4_2958	+	+	+	+	+

Category	System	Gene	Gene products	Role	CDS ^A	Find in				
						KpC4	78578	K2044	Kp13	342
	Ent	<i>entA</i>	2,3-dihydro-2,3-dihydroxybenzoate dehydrogenase		KpC4_2951	+	+	+	+	+

Siderophore based	Fhu	<i>entB</i>	2,3-dihydro-2,3-dihydroxybenzoate synthetase	Enterobactin synthesis	KpC4_2952	+	+	+	+	+
		<i>entC</i>	isochorismate synthase		KpC4_2954	+	+	+	+	+
		<i>entD</i>	enterochelin synthetase component D		KpC4_2964	+	+	+	+	+
		<i>entE</i>	2,3-dihydroxybenzoate-AMP ligase		KpC4_2953	+	+	+	+	+
		<i>entF</i>	ATP-dependent serine activating enzyme	Ferrichrome transport	KpC4_2960	+	+	+	+	+
		<i>fhuA</i>	ferrichrome porin		KpC4_3427	+	+	+	+	+
		<i>fhuB</i>	iron(3+)-hydroxamate import system permease		KpC4_3423	+	+	+	+	+
		<i>fhuC</i>	ferrichrome ABC transporter, ATP-binding protein		KpC4_3425	+	+	+	+	+
	IroA	<i>fhuD</i>	iron(3+)-hydroxamate-binding protein	Salmochelin transport	KpC4_3424	+	+	+	+	+
		<i>iroN</i>	Outer Membrane Siderophore Receptor		KpC4_2314	+	–	+	–	–

Additional Data from Table S3. Continued.

Category	System	Gene	Gene products	Role	CDS ^Δ	Find in				
						KpC4	78578	K2044	Kp13	342
Siderophore based	IroA	<i>iroE</i>	Salmochelin siderophore protein IroE	Salmochelin synthesis	KpC4_1881	+	–	+	–	–
	Aerobactin	<i>iutA</i>	Outer membrane receptor proteins, mostly Fe transport	Aerobactin transport	KpC4_2459	+	±	+	±	±
		<i>iucABCD</i>		Aerobactin synthesis		–	–	+	–	–
	High-pathogenicity island	<i>ybtPQXS, ybtA-irp2-irp1- ybtUTE-fyuA</i>		Yersiniabactin synthesis and transport		–	–	+	+	–

Δ, CDS of the iron scavenging systems system relative to the KpC4 genome; +, gene found with BLASTP identity ≥ 90% and at least 80% coverage. –, gene absent according to the same criteria; ±, a CDS sharing 70% identity is found, although the rest of the system was not identified.

Additional Data from Table S4. The potential targets of *K. pneumoniae* KpC4 Continued.

No.	CDS	Gene	Product	Essentiality	Functional pathway	Proposed inhibitors
1	KpC4-0844	<i>FabB</i>	3-oxoacyl-[acyl-carrier-protein] synthase 1	Yes	Type II fatty acid elongation cycle	TLM, cerulenin
2	KpC4_0324	<i>ArgA</i>	Amino acid acetyltransferase	Yes	Catalyze the first step in L- arginine biosynthesis	
3	KpC4_3397	<i>LpxA</i>	UDP-N-acetylglucosamine O- acyltransferase	Yes	Catalyze the first-step of lipid IV(A) LPS biosynthesis	Compounds 1 and 2
4	KpC4_3497	<i>LpxC</i>	UDP-3-O-[3- hydroxymyristoyl] N- acetylglucosamine deacetylase	Yes	Catalyze the committed step in lipid A LPS biosynthesis	LpxC-2, LpxC-3, LpxC-4
5	KpC4_2478	<i>FabH</i>	3-oxoacyl-[acyl-carrier-protein] synthase 3	No	Catalyze the first condensation reaction in fatty acid biosynthesis	Small molecule inhibitors
6	KpC4_3412	<i>DapD</i>	2,3,4,5-tetrahydropyridine- 2,6-dicarboxylate N- succinyltransferase	Yes	Catalyze the first step of the L-lysine biosynthesis via DAP pathway	L-2- aminopimelate
7	KpC4_3508	<i>MurF</i>	UDP-N-acetylmuramoyl-tripeptide-D-alanyl-D-alanine ligase	Yes	Peptidoglycan biosynthesis	Thiophene-3- carbonitriles
8	KpC4_0724	<i>DapE</i>	Succinyl- diaminopimelate desuccinylase	Yes	Produce intermediates required for L-lysine biosynthesis	L- Captopril
9	KpC4_3399 KpC4_4042	<i>LpxD</i>	UDP-3-O-[3-hydroxymyristoyl] glucosamine N- acyltransferase	Yes	Cell membrane glycolipid biosynthesis	RJPXD33
10	KpC4_2270	<i>FabI</i>	Enoyl-[acyl-carrier-protein] reductase [NADH]	Yes	Enoyl reductase involved in biotin and fatty acids biosynthesis	Diazaborines, triclosan
11	KpC4_3396	<i>LpxB</i>	Lipid- A- disaccharide synthase	Yes	Biosynthesis of lipid A disaccharide	antisense pPNA

Additional Data from Table S4. The potential targets of *K. pneumoniae* KpC4 Continued.

No.	CDS	Gene	Product	Essentiality	Functional pathway	Proposed inhibitors
12	KpC4_3504	<i>MurG</i>	Undecaprenyl-PP-MurNAc- pentapeptide-UDPGlcNAcGlcNAc transferase	Yes	Peptidoglycan biosynthesis	Substituted prolines
13	KpC4_1800	<i>Asd</i>	Aspartate- semialdehyde dehydrogenase	Yes	Biosynthesis of multiple components; L-lysine, L-threonine, L- methionine, L- homoserine	2-Chloro-1,4-naphthoquinone, 2-Bromo-1,4-naphthoquinone
14	KpC4_2425	<i>PurB</i>	Adenylosuccinate lyase	Yes	Biosynthesis of purine nucleotides	-
15	KpC4_2471	<i>Tmk</i>	Thymidylate kinase	Yes	Biosynthesis of pyrimidine deoxyribonucleotides	-
16	KpC4_3504	<i>MurG</i>	UDP-N-acetylglucosamine-N- acetylmuramyl- (pentapeptide) pyrophosphoryl- undecaprenol N- acetylglucosamine transferase	Yes	Cell wall formation and Lipid synthesis	Substituted prolines
17	KpC4_1153	<i>SecA</i>	Protein translocase subunit SecA	Yes	Protein translocation across the bacterial membrane	Azide, decatransin
18	KpC4_3508	<i>MurF</i>	UDP-N-acetylmuramoyl- tripeptide-d-alanyl-d-alanine ligase	Yes	Cell wall formation and murein synthesis	Thiophene-3- carbonitriles, Cyanothiophene derivatives

19	KpC4_4467	<u>WaaO</u>	Lipopolysaccharide core biosynthesis protein	Yes	LPS biosynthesis	-
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Additional Data from Table S4. The potential targets of *K. pneumoniae* KpC4 Continued.

No.	CDS	Gene	Product	Essentiality	Functional pathway	Proposed inhibitors
20	KpC4_1093	<i>SbcB</i>	Exodeoxyribonuclease I	Yes	Degrades single-stranded DNA	-
21	KpC4_0080	<i>TolC</i>	Outer membrane channel protein	Yes	Bacterial efflux system, export antibiotics and other toxic compounds	Domperidone
22	KpC4_3229	<i>YajC</i>	Preprotein translocase subunit	Yes	Protein secretion, insertion of proteins into membranes, and assembly of membrane protein complexes	-