Supplementary materials

Leading region antidefense genes drive conjugation success of IncM and IncL plasmids

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Table S1. Primers used in this study

Primer	Sequence (5´-3´)	Description
Ssb_M_F	AATGGCAAGGGACAGACGGC	For q-RT PCR of IncM plasmid *ssb* gene
Ssb_M_R	AGCACTGGTGTTACGTGGCC	
KlcA_M_F	GCGGTTCCTGGGAGTTCGTCGA	For q-RT PCR of IncM plasmid *klcA* gene
KlcA_M_R	AGCCTTCTTCATGTGCGTGCCA	
HicB_M_F	GTTTGCGCGCCTGAATGAGC	For q-RT PCR of IncM plasmid *HicB*-like gene
HicB_M_R	ACCGCGCTTTCCTCATCGAG	
MTase-1_M_F	GTCTGGAGCCTGGCAAAGCA	For q-RT PCR of IncM plasmid *MTase-1* gene
MTase-1_M_R	CGACTTTTTGCAGCGCCTCG	
Arp_M_F	GATGATGACCTTTGCGCCTCATGC	For q-RT PCR of IncM plasmid *arpA* gene
Arp_M_R	GTCGTCACGCCCGCAACCTGAC	
MTase-2_M_F	GCCCTGCGCGGTAAGAAAAC	For q-RT PCR of IncM plasmid *MTase-2* gene
MTase-2_M_R	CTCGCCTGACATCGCACTGT	
MTase-3_M_F	CGTCAGGAATGGGATCGGCAGG	For q-RT PCR of IncM plasmid *MTase-3* gene
MTase-3_M_R	CTTGCTTGCGTTCAGGTGCCGG	
korC_M_F	ATAAATGGACCCCGCCGACG	For q-RT PCR of IncM plasmid *korC* gene
korC_M_R	TCTGCCGTTCACGCCAACAT	
radC_RT_F	GGGCACTATCAGCAGCGTCACC	For q-RT PCR of IncM plasmid *radC* gene
radC_RT_R	CCTGGCTGGGTGCAGTGTTAGC	
DUF3085_M_F	TGATGGCGGCGAAAGGTGAG	For q-RT PCR of IncM plasmid *DUF3085* domain-containing gene
DUF3085_M_R	CATCACGCATCCGGTCCCAG	
mqsA_M_F	CGTGCATCCTGATTACCGCCTGA	For q-RT PCR of IncM plasmid *mqsA-like* gene
mqsA_M_R	GCCATGACGCCGGTTTATAGCCA	
recA-F	TCCGGTAAAACCACGCTGAC	For q-RT PCR of *E. coli recA* gene
recA-R	CGTGCGTAGATTGGGTCC	
pemI_M_F	GTTATGCTGACCGTTCCACCG	For q-RT PCR of IncM plasmid *pemI* gene
pemI_M_R	TGAATACTGCGGGCGTCTGTGC	
rpoB-RT_F	GTAAGGCACAGTTCGGTGGT	*E. coli rpoB* gene, used to normalise gene relative expression
rpoB-RT_R	ATTTCCTGCAGGGTGTATGC	
rpoB-F	TCGAAGGTTCCGGTATCCTGAGC	core rifampicin-resistant region of *E. coli rpoB*
rpoB_R	GGATACATCTCGTCTTCGTTAAC	
TraY-Exc-FRT-P1	GGCGATGGCTTACTTAATAACGTTTTCTTCACTGTCAACGGTGTAGGCTGGAGCTGCTTCG	Amplification of the Kn-FRT cassette from the pKD4 with homologous arms of *traY* and *exc* genes from the IncM plasmid and used to delete 2430-bp of *traY-exc* gene fragment from IncM plasmid
TraY-Exc-FRT-P2	CCCCGTATTTCGCAGGTTTATGATTAACTCCCTGCCGACTCATATGAATATCCTCCTTA	
LR2.2_Kn_F	AAAATATGTCCCAGAATGAAGCCTATTACGACACGCTGGGTGTAGGCTGGAGCTGCTTCG	Used to amplify the Kn cassette from pKD4 to replace ~2.2-Kb leading region of IncM plasmid pJIMK45
LR5.4_Kn_F	GGCTGATGGGGATCTGAAAGCAAAATTTACAGGGAAAGGGTGTAGGCTGGAGCTGCTTCG	Used to amplify the Kn cassette from pKD4 to replace ~5.4-Kb leading region of IncM plasmid pJIMK45
LR_Kn_R	CAGCCACCCGATATTGTTCACTAATATTCATACTTCCCCCCCATATGAATATCCTCCTTA	Common reverse primer for the amplification with the previous two forward primers

Table S2. Plasmids used in this study

Plasmid	Description	Source/Reference
pJIBE401	Naturally occurring IncM plasmid carrying bla_{IMP-4} carbapenemase gene	(1)
pJIMK45	Derivative of pJIBE401. The ~28-kb multi-resistance region of pJIBE401 was replaced by tetracycline resistance gene *tetA*	(1)
pJIMK45ΔTraY-ExcA	*traY* and *excA* genes have been deleted from the pJIMK45 by inserting the kanamycin-resistant marker gene *aphA*	This study
pJIMK45Δ2.2LR	A ~2.2 kb leading region was replaced by the kanamycin resistance gene *aphA*	This study
pJIMK45Δ5.4LR	A ~5.4 kb leading region was replaced by the kanamycin resistance gene *aphA*	This study
pKD4	Kanamycin-resistant cassette-carrying plasmid	(2)
pKM200	Lambda red recombinase plasmid	(3)

Table S3. Bacterial strains used in this study.

Strain name	Genotype or characteristics	Source/Reference
J53Az	Derivative of *E. coli* K-12, plasmid-free, Sodium azide resistant	(4)
UB5201Rf	Derivative of *E. coli* K-12, plasmid-free, RIF resistant	(5-7)
NH78Rf	RIF resistant clinical *E. coli* ST131 (RpoB D516G)	This study
JIE286	*E. coli* ST131 isolate, carrying pJIE286_F ($bla_{CTX\text{-}M\text{-}15}$) and pJIE286_Col	(8)
JIE286C	Antibiotic sensitive derivative of JIE286, cured of pJIE286_F, carrying pJIE286_Col	This study
JIE286CRf	RIF resistant derivative of JIE286C (RpoB S512P), carrying pJIE286_Col	This study
WH62Rf	WH62 is an antibiotic-sensitive clinical *E. coli,* and WH62Rf is the RIF-resistant derivative (RpoB S522Y) of WH62	This study
Nissle1917Str	Streptomycin-resistant derivative of Nissle1917 probiotic *E. coli*	(9)
WH49Rf	WH49 is an antibiotic-sensitive clinical *K. pneumoniae* isolate, and WH49Rf is the RIF-resistant derivative (RpoB L511Q) of WH49	This study
NH92Rf	NH92 is an antibiotic-sensitive clinical *K. pneumoniae* isolate, and NH92Rf is the RIF-resistant derivative (RpoB S512P) of NH92	This study
WH82Rf	WH82 is an antibiotic-sensitive clinical *K. pneumoniae* isolate, and WH82Rf is the RIF-resistant derivative (RpoB S531F) of WH82	This study
KpATCC13883Rf	RIF-resistant derivative of *K. pneumoniae* ATCC strain	(10)
Mm1585Rf	RIF-resistant derivative (RpoB D516Y) of *Morganella morganii* isolate	(10)

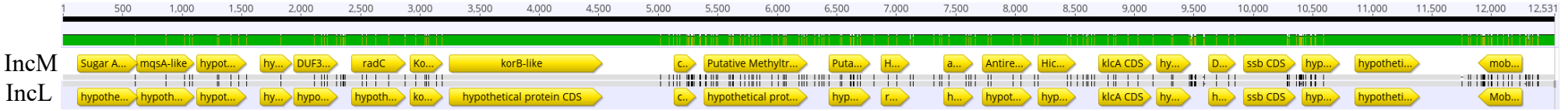

Fig S1. Nucleotide sequence alignment between IncM and IncL plasmids leading regions. Black bars represent non-identical nucleotide sequences.

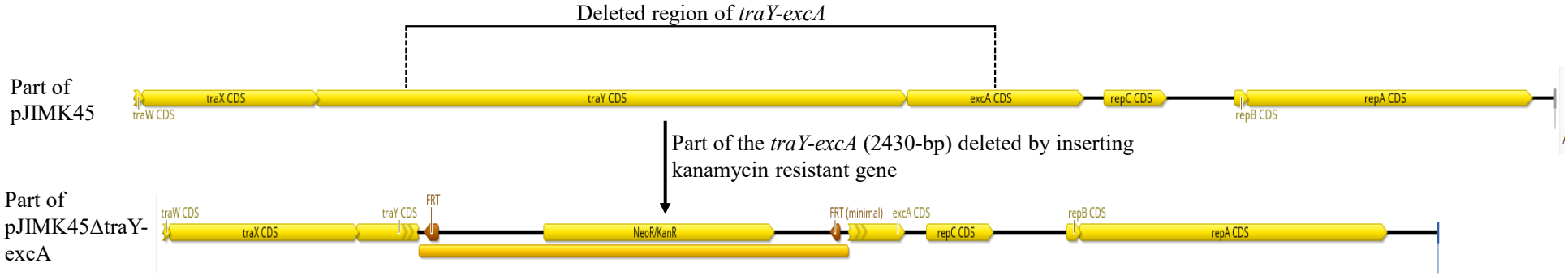

Fig S2. IncM plasmid derivative pJIMK45ΔtraY-excA has been constructed by deleting the part of *traY-excA* region from the pJIMK45 plasmid.

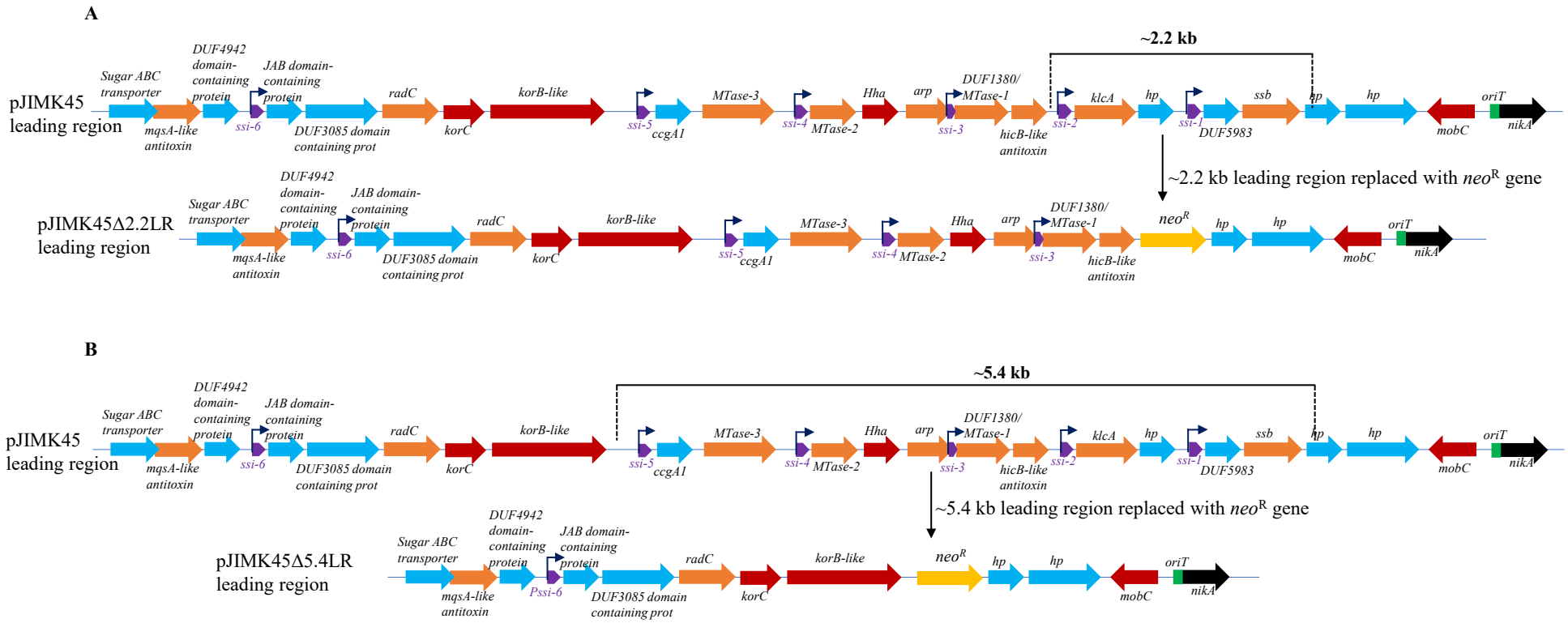

Fig S3. IncM plasmid leading region deletion mutants. (**A**) a ~2.2 kb leading region was deleted from the IncM plasmid pJIMK45 by inserting a neomycin resistance gene. (**B**) a ~5.4 kb leading region (contains previous ~2.2 kb and additional ~3.2 kb) of IncM plasmid pJIMK45. The orange arrows are for putative anti-defense genes, the red arrows represent regulatory genes, and the blue arrows denote genes with unknown functions. Six single-strand-specific promoter regions are shown by small purple arrows (ssi1-6).

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