Supplemental Material

The role of long and short replication initiation proteins in the fate of IncP-1 plasmids

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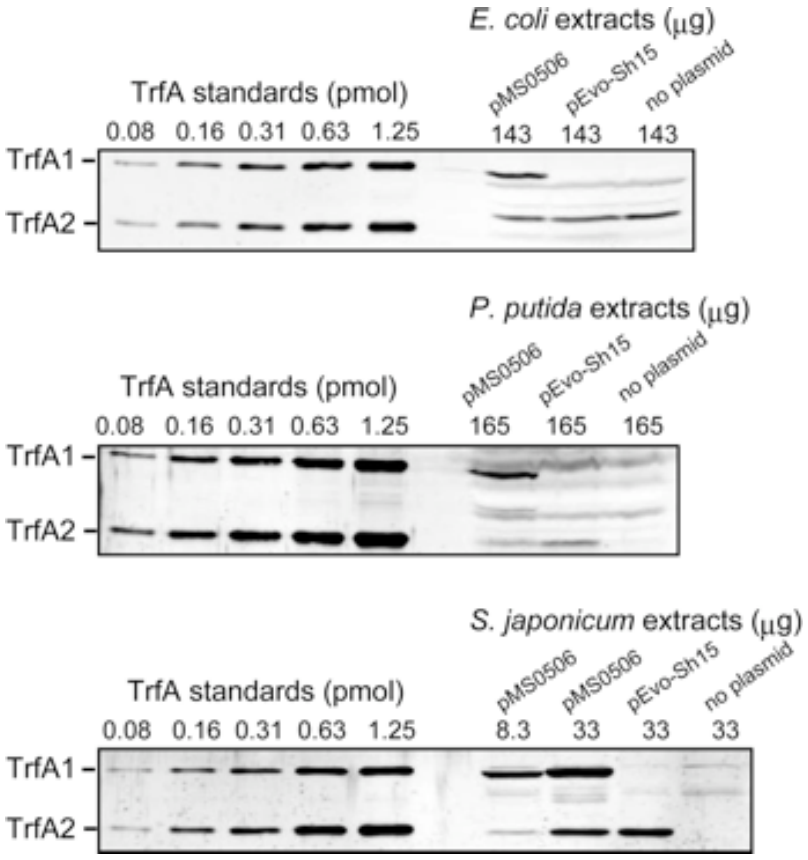

Fig. S1. Effect of the frame-shift mutation in pEvo-Sh15 on production of TrfA1 and -2. Quantity of cell extracts was normalized for total protein content using the Bradford assay. Individual TrfA levels are summarized in Table S2.

Table S1. Oligonucleotides used in this study.

Name	Sequence (5' – 3')	Purpose
SjaporiCF	CGTCAACATCATCGTAACCG	Cloning of the SJA-00140 region of the *S. japonicum* chromosome I
SjaporiCR	TGAAGATATTTTGGCCCCAG	Cloning of the SJA-00140 region of the *S. japonicum* chromosome I
EcolioriCF	TACGGCATCCTGAGGGAATTCGA	Cloning of the *atpB* region of the *E. coli* chromosome
E.colioriCR	GTCTTAAGGGGACTGGAGCATGCA	Cloning of the *atpB* region of the *E. coli* chromosome
PpuoriCF	GGTGACGAATTCCAGCAGGAA	Cloning of the *atpB-parB* region of the *P. putida* chromosome
PpuoriCR	CTGCAACAGGAATTCGAACTCACC	Cloning of the *atpB-parB* region of the *P. putida* chromosome
JMPoriCF	TCACGTTCTGCGTCAAAATC	Cloning of the *parB* region of the *C. necator* chromosome I
JMPoriCR	GATCCGCGAATTCCAGTTTA	Cloning of the *parB* region of the *C. necator* chromosome I
pBP136kleEF	CGTCTCGATATACAAGCCCA	qPCR, amplification of the 158 bp fragment in *kleE* of pBP136
pBP136kleER	ATTGCACCCATAACACTCCA	qPCR, amplification of the 158 bp fragment in *kleE* of pBP136
tetAF	GTGAAACCCAACATACCCCT	qPCR, amplification of 170 bp fragment in *tetA* of pHY872 derivatives
tetAR	AATTGCACCAACGCATACAG	qPCR, amplification of 170 bp fragment in *tetA* of pHY872 derivatives
EcoliatpF	GTCGGTCCAGGTCTTCATTT	qPCR, amplification of the 157 bp fragment in *atpB* of *E. coli*
EcoliatpR	TGCACACGGTAATCTGGAAT	qPCR, amplification of the 157 bp fragment in *atpB* of *E. coli*
PpuparBF	CGCAGAGTATAGGGGTGGAT	qPCR, amplification of the 150 bp fragment in *parB* of *P. putida*
PpuparBR	GCAAAGGCCAGTTGGTTATT	qPCR, amplification of the 150 bp fragment in *atpB* of *P. putida*
Sjap0140F	TCAACGACAAGATCAGCGAC	qPCR, amplification of the 131 bp fragment in *parB* of *S. japonicum* chromosome I
Sjap0140R	GAAGCGGTCGAGATTGTAGC	qPCR, amplification of the 131 bp fragment in *parB* of *S. japonicum* chromosome I
parBJMPF	CGACAATTCTTCTTCCAGCC	qPCR, amplification of the 134 bp fragment in *parB* of *C. necator* chromosome I
parBJMPR	TCAACAAGCGGCTATCAGTG	qPCR, amplification of the 134 bp fragment in *parB* of *C. necator* chromosome I
SacI-SD-pBP136trfA1F	AATTGAGCTCAAGGAGGTAATACACCATGACGAACAACGAGTTCAACGA	Cloning of *trfA1* into pHSG399
SacI-SD-pBP136trfA2F	AATTGAGCTCAAGGAGGTAATACACCATGGCGACCAAGAAGCGAAC	Cloning of *trfA1* and *trfA2* into pHSG399
Hind-pBP136trfAR	AATTAAGCTTATTACCGCTTGCAATGCACCAGGT	Cloning of *trfA2* to pHSG399
trfAM124LF	CTGGCGACCAAGAAGCGAACG	Introducing M124L mutation to *trfA1*
trfAM124LR	CGCACTACTCCGTTTGTCCTG	Introducing M124L mutation to *trfA1*
trfA1-F-NdeI	TTTTCATATGACGAACAACGAGTTCAA	Cloning of *trfA1* into pTXB1
trfA2-F-NdeI	TTTTCATATGGCGACCAAGAAGCGAAC	Cloning of *trfA2* into pTXB1
trfA-R-Xho	TTTTCTCGAGTCACCGCTTGCAATGCACCA	Cloning of *trfA1* and *trfA2* into pTXB1
Eco-pBP136iteron8R	AATTGAATTCCGGGCTTGTGGATCGTTTG	Cloning of $oriV_{pBP136}$ to pJP5608
Sph-pBP136oriVF	AATTGCATGCCGGGTGGGATCGAGAAGGG	Cloning of $oriV_{pBP136}$ to pJP5608
Sph-pJPlacPdel	AATTGCATGCCGCTCGGCCAGTCCCTG	Deletion of *lac* promoter on the pJP5608 derivative

Tn7R109[a]	CAGCATAACTGGACTGATTTCAG	Confirmation of Tn*7* insertion to attTn*7 of E.coli*, *P. putida*, *S. japonicum*, *C. necator*
EcoliglmS	CATGCACATCATCGAGATGCC	Confirmation of Tn*7* insertion to attTn*7* of *E. coli*
PseglmS[a]	AATCTGGCCAAGTCGGTGAC	Confirmation of Tn*7* insertion into attTn*7* of *P. putida*
Sjap_glmSDN	CATGGCGACCATCACCATGC	Confirmation of Tn*7* insertion into attTn*7* of *S. japonicum*
Cupriavid_glmSDN	AACCTGGCGAAGTCGGTGAC	Confirmation of Tn*7* insertion into attTn*7* of *C. necator*
pBAD24CmF	TGAGACAATAACCCTGATAAATGCTTCA ATAATATTGAAAAAGGAAGAGTATGGAG AAAAAAATCACTGGATATAC	Construction of pBAD24C
pBAD24CmR	AGGGCGCGTAAATCAATCTAAAGTATAT ATGAGTAAACTTGGTCTGACAGTCATCGC AGTACTGTTGTATTC	Construction of pBAD24C
EcopBPTrfA2F	AATTGAATTCACCATGGCGACCAAGAAG CGAAC	Cloning of *trfA2* to pBAD24C
EcopBPTrfA1F	AATTGAATTCACCATGACGAACAACGAG TTCAACGA	Cloning of *trfA1* to pBAD24C
HindpBPTrfAR	AATTAAGCTTATTACCGCTTGCAATGCAC CAGGT	Cloning of *trfA1* and *trfA2* to pBAD24C

[a] Primers, Tn7R109 and PseglmS were identical to the primers reported by Lambertsen et al. (2004) in *Environ. Microbiol.* Vol 6: 726-732.

Table S2. TrfA levels[a] in three hosts harboring pMS0506 or pEvo-Sh15 in exponential phase.

	TrfA1 (fmol/ug)	TrfA2 (fmol/ug)	Total TrfA (fmol/ug)	Relative total TrfA levels	Number of samples
E. coli BW25113					
pMS0506	2.19	<0.2	2.2	1.00	N=7
pEvo-Sh15	NA[b]	<0.5	<0.5	<0.22	N=4
P. putida KT2440					
pMS0506	2.09	0.23	2.3	1.00	N=6
pEvo-Sh15	NA	0.48	0.5	0.21	N=4
S. japonicum UT26S					
pMS0506	39.84	12.52	52	1.00	N=5
pEvo-Sh15	NA	12.61	13	0.24	N=1

[a] Mean values are shown. Examples of Western blots are shown in Fig. S1.

[b] NA, not applicable