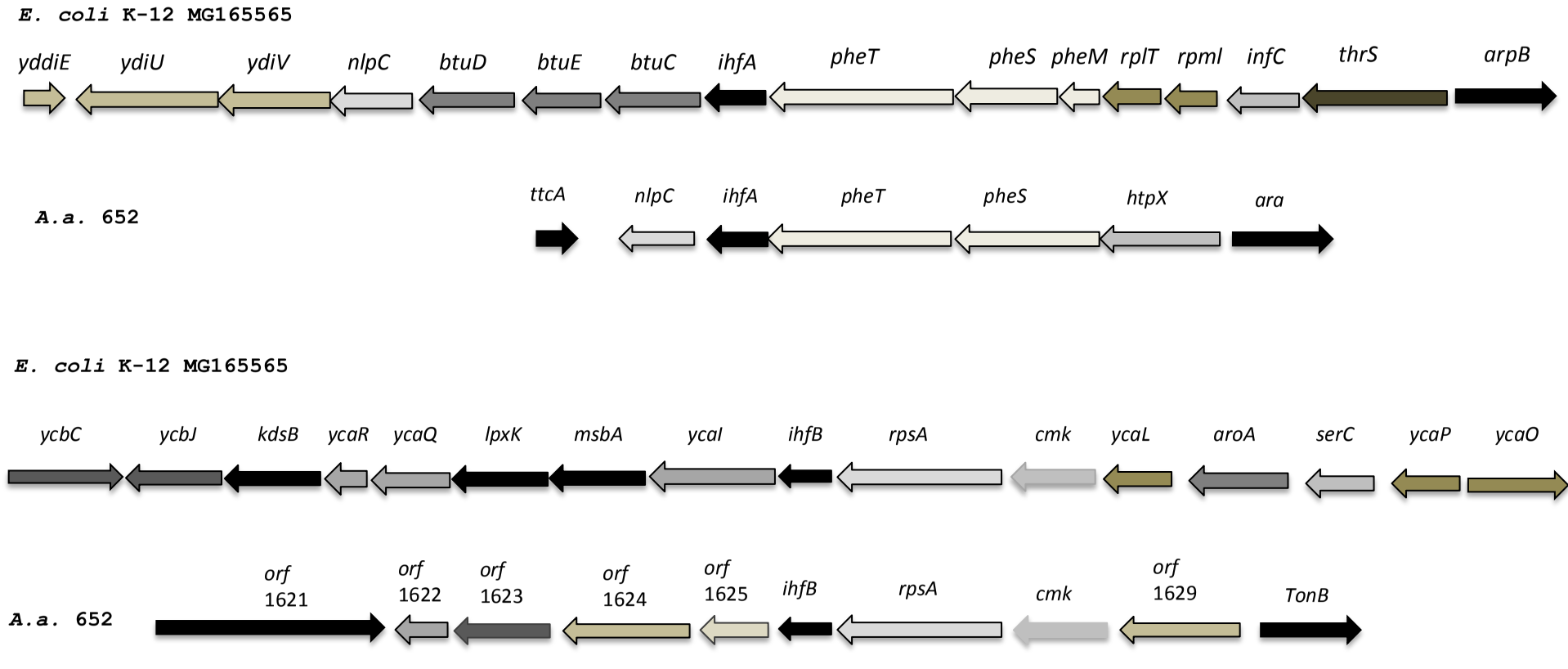
Figure S1

A



B

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A. a. IHFα   3   LTKVELAENLIEK-FHLSKREAKDLVESFFEEIRVALETGNDVKLSGFGNFELRDKASRP  61
                 +TK EL E L++K  ++  +  ++ V++  E++   LE G  +++ GFG+F L  +  R
A. a. IHFβ   1   MTKSELIELLVQKNSNIPVKHVEEAVKAILEQMSYVLEHGERIEVRGFGSFSLHCRQPRI  60

A. a. IHFα   62  GRNPKTGESVPVSARRVVVFKPGQKLRNRVE  92
                 GRNPKTGE V + A+ V  FK G++LR RV+
A. a. IHFβ   61  GRNPKTGEQVKLDAKCVPYFKAGKELRERVD  91
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Figure S1. (A) Genomic organization of the i*hfA* and *ihfB* loci in *A. actinomycetemcomitans* compared to the related loci in *Escherichia coli*. (**B**) Protein sequence alignment of *A. actinomycetemcomitans* IHFα and IHFβ. Residue numbers are shown to the right of each protein sequence.

Table S1. Strains and plasmids used in this work

Strain or plasmid	Derived, relevant genotype[a] or characteristics[b]	Source or reference
Escherichia coli		
XL1-Blue MRF'	Δ(*mcrA*)*183* Δ(*mcrCB-hsdSMR-mrr*)173 *endA1 supE44 thi-1 recA1 gyrA96 relA1 lac* [F´ *proAB*. *lacI*^{q}ZΔ*M15* Tn10 (Tc^{r})]	Stratagene
LMG194	F^{-} Δ(*lacIPOZY*)X74 *galE galK thi rpsL* Δ*phoA* (*Pvu*II) *ara*714 *leu*::Tn*10*	Invitrogen
BL21	F^{-} *ompT hsdS*$_B$(r_B^{-} m_B^{-})*gal dcm rne131* (DE3)	Novagen
Top10	F^{-} *mcrA* Δ(*mrr-hsd*RMS-*mcrBC*) Φ80*lacZ*ΔM15 Δl*ac*X74 *recA1 araD*139 Δ(*ara leu*) 7697 *galU galK rpsL* (Str^{r}) *endA1 nupG*	Invitrogen
Aggregatibacter actinomycetemcomitans		
652	Wild type, serotype c	(30)
652-TE78	652, Δ*ihfA*	This study
652-TE79	652, Δ*ihfB*	This study
652-TE-23	652, *lsrR::lsrR23-lacZ*	This study
652-TE78-23	652, Δ*ihfA lsrR::lsrR23-lacZ* Sp^{r}	This study
652-TE79-23	652, Δ*ihfB lsrR::lsrR23-lacZ* Sp^{r}	This study
652-TE-75	652, *lsrA::lsrA75-lacZ*	This study

652-TE78-75	652, Δ*ihfA lsrA::lsrA75-lacZ* Sp^r	This study
652-TE79-75	652, Δ*ihfB lsrA::lsrA75-lacZ* Sp^r	This study
Plasmid		
pYA3883	Amp^r, expression vector	(48)
pET28a+	Km^r, pBR322 expression vector	Novagen
pJT3	Km^r, *lacZ* promoterless	(47)
pJT7	pYGK, Km^r, cloning vector	Torres-Escobar et al., submitted
pJT1	Sp^r, suicide vector	(47)
pJT10	Sp^r, suicide vector	Torres-Escobar et al., submitted
Derived from pYA3883		
pATE76	Amp^r, P_{BAD}*ihfA*-(AUI)-(6X His)	This study
pATE77	Amp^r, P_{BAD}*ihfB*-(AUI)-(6X His)	This study
Derived from pET28a+		
pATE80	Km^r, pET28a+ *ihfB*-(AUI)-(6X His)	This study
Derived from pJT1		
pATE78	Sp^r, flanking region to *ihfA*	This Study
pATE79	Sp^r, flanking region to *ihfB*	This Study
Derived from pJT3		
pATE23	Km^r, $P_{lsrR\ -1\ to\ 255}$-*lacZ*	(30)
pATE68	Km^r, $P_{lsrR\ -82\ to\ -255}$-*lacZ*	(30)
pATE69	Km^r, $P_{lsrR\ -1\ to\ 193}$-*lacZ*	This study
pATE70	Km^r, $P_{lsrR\ -1\ to\ 143}$-*lacZ*	This study
pATE100	Km^r, $P_{lsrR\ -1\ to\ 414}$-*lacZ*	This study
pATE71	Km^r, $P_{lsrA\ -1\ to\ 249}$-*lacZ*	(30)
pATE73	Km^r, $P_{lsrA\text{-}88\ to\ 414}$-*lacZ*	This study
pATE74	Km^r, $P_{lsrA\ -43\ to\ 414}$-*lacZ*	This study

pATE75	Km^r, $P_{lsrA\text{-}1\ to\ 414}$-*lacZ*	This study
Derived from pJT7		
pATE92	Km^r, P_{ihf}-*infA*	This study
pATE93	Km^r, , P_{ihf}-*infB*	This study
Derived from pJT10		
pATE94	Sp^r, *lsrR::lsrR23-lacZ*	This study
pATE95	Sp^r, *lsrA::lsrA75-lacZ*	This study

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[a] In the descriptions of the genotype, P represents promoter, and the subscript refers to the promoter fragment driving *lacZ* transcription. For consistency, nucleotide -1 is the first base upstream from the *lsrR* or *lsrA* start codon. [b] Amp^r ampicillin resistance, Km^r kanamycin resistance, Sp^r spectinomycin resistance, Tc^r tetracycline resistance.

TABLE S2. Oligonucleotides used in this work

Related product, name	Sequence 5’ - 3’
Upstream region *lsrR*	
ATE-6R *Bam*HI	CTTGATTATCCG TGGATCCCATTAATTCGTTC
ATE-172F *KpnI*	TCAAAATACTAGGGTACCTCAGGAATGCACAAAATT TC
ATE-173F *Kpn*I	TCTGGATAATATGGTACCTATCTAAAAATCGCCT
ATE-242F *kpn*I	GCCGGCGATAATTTTCATGGTACCAGCGTGGATTTCCCCGCCCCG
Upstream region *lsrA*	
ATE-7F *Kpn*I	CTTGATTATCCGTGGTACCCATTAATTCGTTC
ATE-165R *Bam*HI	GCTTTCAGCACTTCGGATCCCATGCCGGAAAACG
ATE-166F *Bam*HI	CGCCGAGCAGTGCGTGGGATCCCATTTCACCGGC
***ihfA*-AUI-6His**	
ATE-188F *Nco*I	GCTTATTTGAGAGATTAAACCATGGCATTAACTAAAGTAG
ATE-189R *Apa*I	CAGTAGCCAATTTATTAGGGCCCAGCTTTCGGTTTGACC
***ihfB*-AUI-6His**	
ATE-190F *Nco*I	GAGTTAGGAGAATCCATGGCAAAGTCAGAACTTATTG
ATE-191R *Apa*I	CCGTTATGAAAAGTTAGTTAGTAAAAAATTAGGGCCCTGCTGCATAAACGTC
ATE192F *Nco*I	CAGGAGGAATTAACCATGGCAAAGTCAGAACTTATTGAAC
ATE193R *Xho*I	CTCGTTATTTAATGATGATGATGATGATGCTCGAGGATGTAGCGGTACGTGTC

Deletion of *ihfA*

ATE-157F *Nhe*I	GGACGAAAAGGCCGCTAGCGGCGTGCACCAAG
ATE-134R *Xho*I	CGAGTTCTACTTTAGTTAATGTCATCTCGAGAGTTTAATCTCTC
ATE-135F *Xho*I	CAAACCGAAAGCTTAACTCGAGTAAATTGGCTACTGC
ATE-158R *Sac*I	CTAGAAATTGTATGTGAGAGCTCTTAAATTCGTCTTGCC

Deletion of *ihfB*

ATE-159F *Nhe*I	CAAACCTAACGCTAGCACACAATTCGCCGAAACCC
ATE-142R *Xho*I	CAATAAGTTCTGACTTTGTCATCTCGAGATATTCTCCTAACTC
ATE-143F *Xho*I	GACGTTTATGCAGCATAACTCGAGTTTTTTACTAACTAACTTTTCATAACGGC
ATE-160R *Sac*I	CGGAGAGACTTTAGAGCTCTGCAATAAGATTTGCTG

PCR verification of Δ*ihfA*

ATE137	CAGCAGGCGGAGATAAATTAACCC
ATE140	CTGTTCGCTTAAAAGCGCAATCGCC

PCR verification of Δ*ihfB*

ATE145	GCCCGTATCGTTCATTTATCTGTTCG
ATE148	GGTAGAAAGCTGGAATTGGCTTTGCG

Cloning *IhfA*

ATE-216F *Kpn*I	GGTCGCACACATTTCTCGGTACCGAGAAGTCGCCCGTG
ATE-218R *Xba*I	CGCCCATTCGTATTGCTGTTCGCTTCTAGAAAAGCGCAATCGCC

Cloning *ihfB*

ATE-219F *Kpn*I	GGCTGATTTAACTAACGAAGTGGTACCTTGCCGGCGATG

ATE-221R *Xba*I	GGTAATGATTTGATCATTGTTCTAGAATGGTAACGGCAACCAG

EMSA

lsr f I-84	GTGCGGTCAGTTTTTCCGATG
lsr r I-85	CGCCGGCATTCAGCCACAAG
lsr f II-68	ATTTTCCTTCACCGTTAAATTCGG
lsr r II-69	ACGCACTGCTCGGCGGTAAC
lsr f III-62	GAGCTGATGGGCTTTATTCG
lsr r III-64	GCCCGAAACATCGGTAAGTCG
lsr f IV-74	GCCGGCGATAATTTTCATCAG
lsr r IV-75	CATTTGTTCATTCTATGCGGC
lsr f V-70	GCACTTCACCGGCATATAAG
lsr r V-71	GAAATTTTGTGCATTCCTGAC
lsr f VI-78	GGAAAAATCAATGGCTTTCAG
lsr r VI-79	CCAGATTTGTGATCTAGTTCTCA
lsr f VII-63	CGACTTACCGATGTTTCGGGC
lsr r VII-66	GTAAAAGCATGATGGAGATCACT
lsr f VIII-80	GATATTAAGCATTTTGGCTCTG
lsr r VIII-81	GTTTTTTATTATTATCAATTGTTCTTACCG
lsr f IX-76	GCCGCATAGAATGAACAAATG
lsr r IX-77	GATTTTGGGTATTATTCATTTG
lsr f X-72	TGAGAACTAGATCACAAATCTGG
lsr r X-73	CTTTGGATCGTTTCTTGATTATCC
lsr f XI-65	AGTGATCTCCATCATGCTTTTAC
lsr r XI-67	CAGGTTATCGTGATAATAAAACC

lsr f XII-82	GAACGAATTAATGACGGATAATCAAG
lsr r XII-83	GAATTGATCTGGACTTTAATAATGCCTG
lsr f Yp-98	CCATGGAGACTGTTCTCATG
lsr r Yp-99	CATCACCCGGCTGTTTACCCGC

Gene Racer

ATE-208R	GCATCTGATTCGGTTTAAGCAACGACATCAGCATTTGTG
ATE-210R	ATAAGCCCACGCATAATTTCCACCAGCTGTTGATCGGCAAC

PCR verification of integration

ATE-208R	GCATCTGATTCGGTTTAAGCAACGACATCAGCATTTGTG
ATE-210R	ATAAGCCCACGCATAATTTCCACCAGCTGTTGATCGGCAAC
MDJR-123R	GGGTTTTCCCAGTCACGACGTTGTAAAACGAC
MDJR-148F	GGTCTGACAGTCGACTTTCGTTCGTGAATACATG