SUPPLEMENTARY FIGURES AND TABLES

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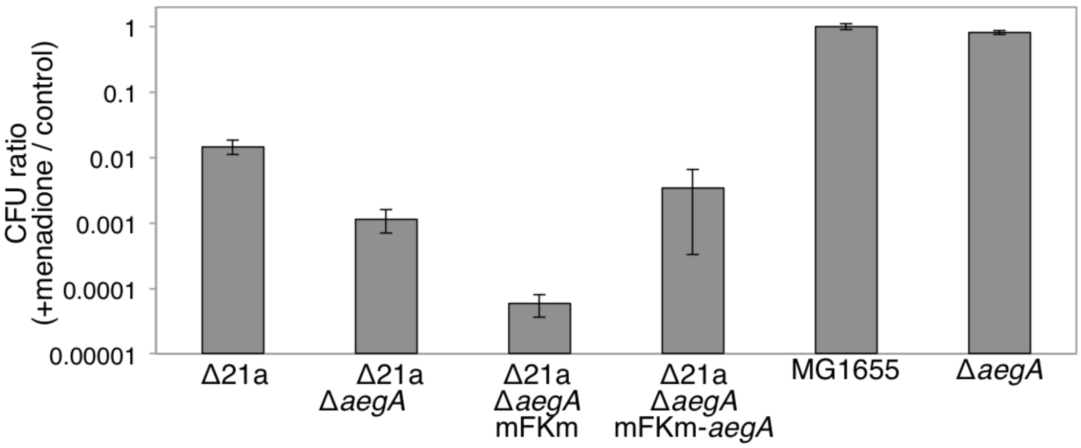

FIG S1 Menadione sensitivity of the *aegA* mutant of a reduced-genome *E. coli* strain and wild-type *E. coli*. Menadione sensitivity of strain Δ21 (a genome-reduced strain), Δ21Δ*aegA* strain, wild-type MG1655 strain, and Δ*aegA* strain was determined.

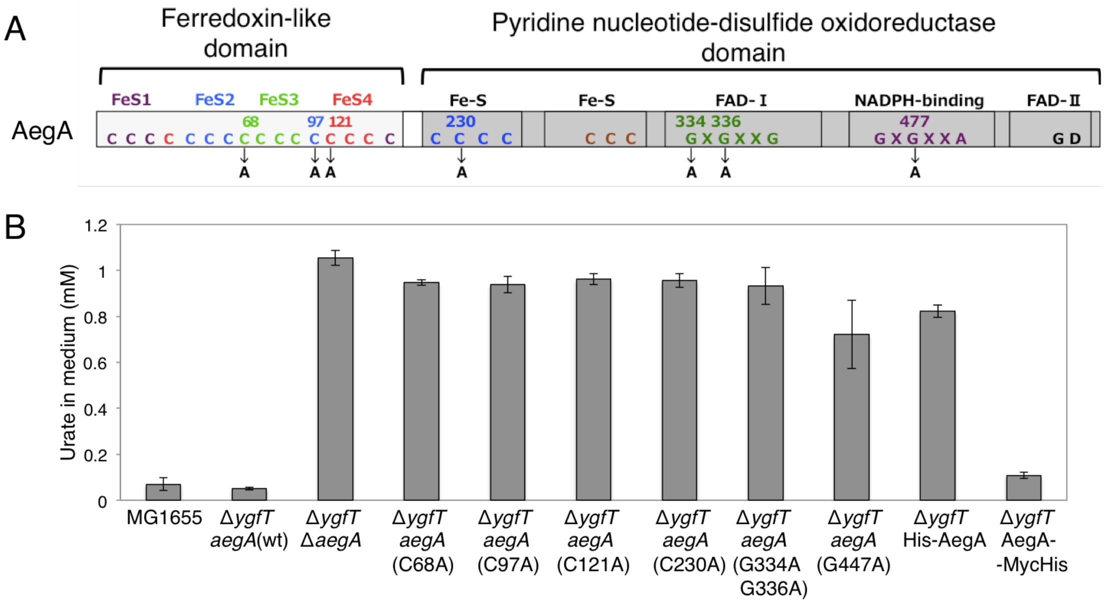

FIG S2 The effect of substitution of conserved amino acid residues of AegA on urate-degrating activity. (A) Schematic representation of the conserved amino acids at the Fe-S sites in the N-terminal ferredoxin-like domain, and the Fe-S sites, FAD-binding site, and NADPH-binding site in the C-terminal pyridine nucleotide-disulfide oxidoreductase domain, of AegA. (B) The effect of substitution of C68, C97, C121, C230, G334G336, and G447 to A on the cellular uric acid degradation activity.

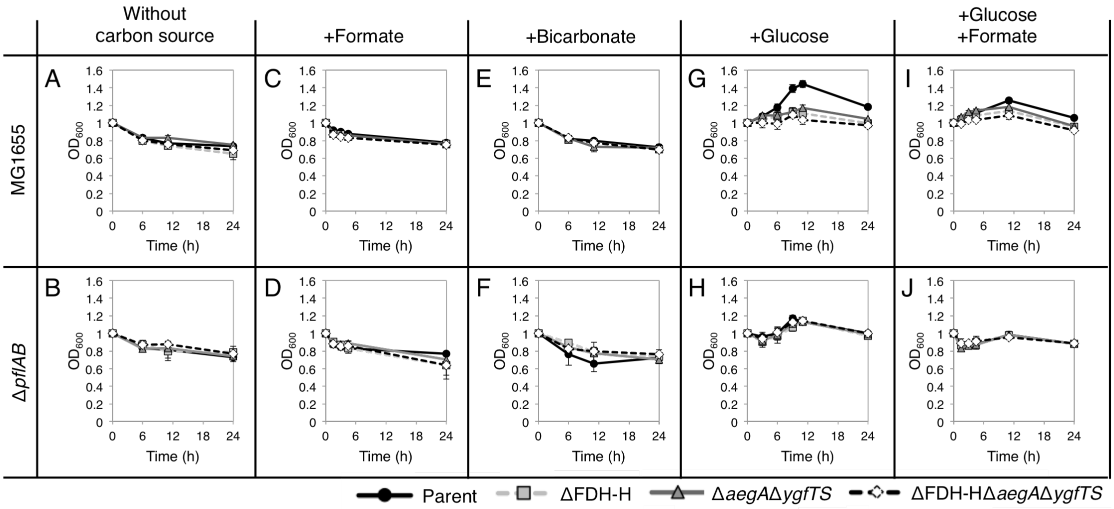

FIG S3 The effect of various carbon sources on cell growth. Cell growth in uric acid in unsupplemented minimal medium (without a carbon source) during a 24 h incubation of (A) wild-type derivatives and (B) Δ*pflAB* derivatives. The effect of the supplementation of a minimal medium with formate on the growth on uric acid of wild-type derivatives (C) and Δ*pflAB* derivatives (D) during a 24 h incubation. The effect of the supplementation of a minimal medium with bicarbonate on the growth on uric acid of wild-type derivatives (E) and Δ*pflAB* derivatives (F) during a 24 h incubation. The effect of the supplementation of a minimal medium with glucose on the growth on uric acid of wild-type derivatives (G) and Δ*pflAB* derivatives (H) during a 24 h incubation. The effect of the supplementation of a minimal medium with glucose and formate on the growth on uric acid of wild-type derivatives (I) and Δ*pflAB* derivatives (J) during a 24 h incubation.

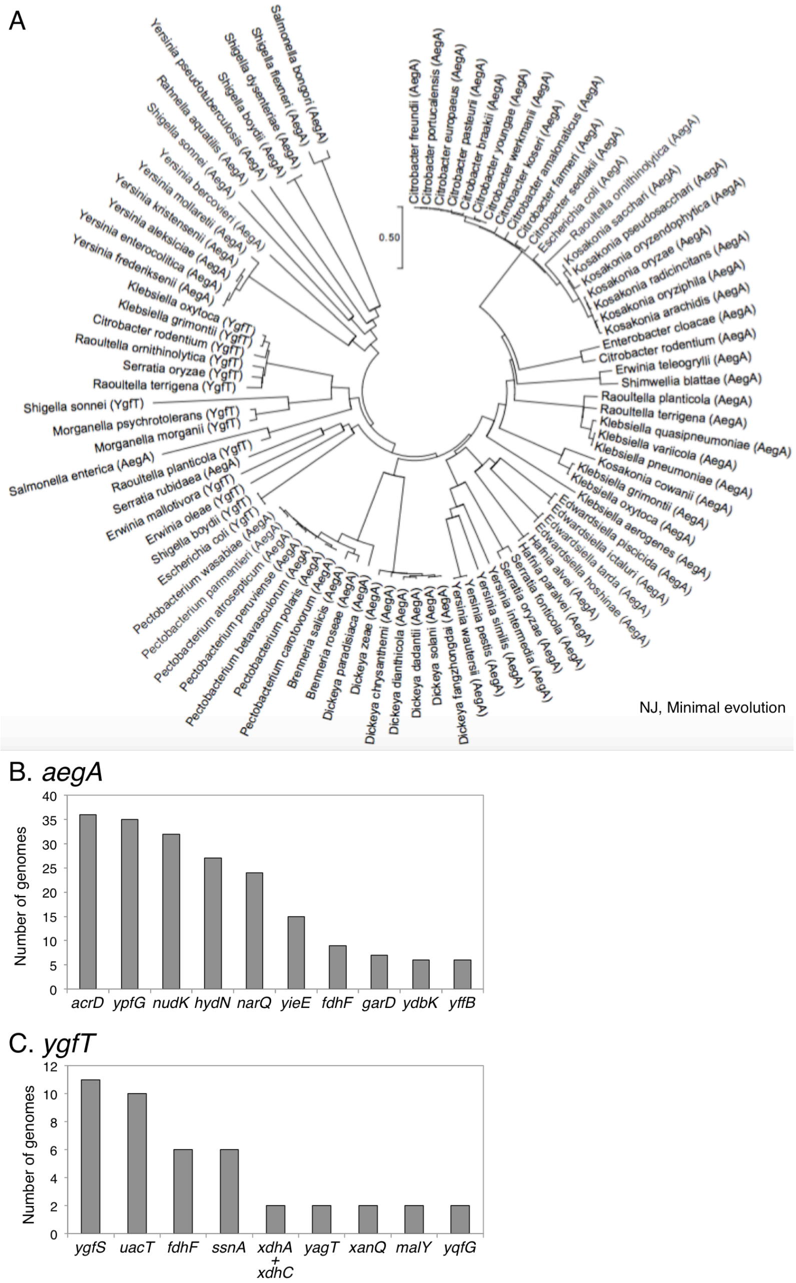

FIG S4 Taxonomic distribution of AegA and YgfT. (A) Taxonomic distribution of AegA and YgfT is shown. Network analysis of proteins encoded by the flanking genes (± 2 genes) of *aegA* (B) and *ygfT* (C) was performed. Top-10 most frequently found genes are shown.

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AegA        1   MNRFIMANSQQCLGCHACEIACVMAHNDEQHVLSQHHFHPRITVIKHQQQRSAVTCHHCEDAPCARSCPNGAISHVDDSI    80
YgfT        1   MNKFIAAEAAECIGCHACEIACAVAHNQENWPLSHSDFRPRIHVVGKGQAANPVACHHCNNAPCVTACPVNALTFQSDSV    80
PfNfn1L

AegA       81   QVNQQKCIGCKSCVVACPFGTMQIVLT[9]TAHKCDLCAGRENGP-ACVENCPADALQLVTDVALSGMAKSRRLRTARQE   165
YgfT       81   QLDEQKCIGCKRCAIACPFGVVEMVDT   IAQKCDLCNQRSSGTqACIEVCPTQALRLMDDKGLQQIKVARQRKTAAGK   157
PfNfn1L

AegA      166   [5]ASTAAQEmpvMSKVEQMQATPARGEPDKLAIEARKTGFDEIYLPFRADQAQREASRCLKCG-EHSVCEWTCPLHNHI   246
YgfT      158      ASSDAQP----SRSAALLPVNSRKGADKISASERKTHFGEIYCGLDPQQATYESDRCVYCA-EKANCNWHCPLHNAI   229
PfNfn1L     1   -----------------MPRLIKDRVPTPERSVGERVRDFGEVNLGYSWELALREAERCLQCPvEYAPCIKGCPVHINI    62

AegA      247   PQWIELVKAGN   IDAAVELSHQTNTLPEITGRVCPQDRLCEGACTIRDEHGAVTIGNIERYISDQALAKG   WRPDL   320
YgfT      238   PDYIRLVQEGK   IIEAAELCHQTSSLPEICGRVCPQDRLCEGACTLKDHSGAVSIGNLERYITDTALAMG   WRPDV   303
PfNfn1L    63   PGFIKALRENR[6]VREALRIIWRDNTLPAITGRVCPQEEQCEGACVVGKVGDPINIGKLERFVADYAREHG[5]LLEEI   147

AegA      321   SHVTKVDKRVAIIGAGPAGLACADVLTRNGVGVTVYDRHPEIGGLLTFGIPSFKLDKSLLARRREIFSAMGIHFELNCEV   400
YgfT      304   SKVVPRSEKVAVIGAGPAGLGCADILARAGVQVDVFDRHPEIGGMLTFGIPPFKLDKTVLSQRREIFTAMGIDFHLNCEI   383
PfNfn1L   148   KGIKRNGKKVAIIGAGPAGLTCAADLAKMGYEVTIYEALHQPGGVLIYGIPEFRLPKEIVKKELENLRRLGVKIETNVLV   227

AegA      481   MDCVRTALRHGASNVTCAYRRDEANMPGSKKEVKNAREEGANFEFNVQPVALELNEQGHVCGIRFLRTRLGEPDAQGRRR   480
YgfT      464   MDCLRTSIRLNAASVTCAYRRDEVSMPGSRKEVVNAREEGVEFQFNVQPQYIACDEDGRLTAVGLIRTAMGEPGPDGRRR   463
PfNfn1L   306   MDAARSALRLG-AEVWILYRRTRKEMTAREEEIKHAEEEGVKFMFLVTPKRFIGDENGNLKAIELEKMKLGEPDESGRRR   305

AegA      561   PVPVEGSEFVMPADAVIMAFGFNPHGMPWLESHGVTVDKWGRIIADVESQYRYQTTNPKIFAGGDAVRGADLVVTAMAEG   640
YgfT      544   PRPVAGSEFELPADVLIMAFGFQAHAMPWLQGSGIKLDKWGLIQTGDVGYLPTQTHLKKVFAGGDAVHGADLVVTAMAAG   623
PfNfn1L   385   PIPT-GETFIMEFDTAIIAIGQTPNKTFLETVPGLKVDEWGRIVVDEN----LMTSIPGVFAGGDAIRGEATVILAMGDG   459

AegA      641   RHAAQGIIDWLGVKSVKSH                                                                659
YgfT      624   RQAAR---DMLTLFDTKAS                                                                639
PfNfn1L   468   RKAAKAIHQYLSKEK----                                                                474
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FIG S5 Comparison of amino acid sequences for AegA/YgfT and a bifurcating enzyme NfnI-L. Multiple-sequence alignment was made using *Cobalt* (1). The amino acid residues in red represent identical amino acids, and amino acid residues in blue represent similar amino acids, in the three compared sequences. Important residues close to the FAD-binding site in NfnI-L are boxed.

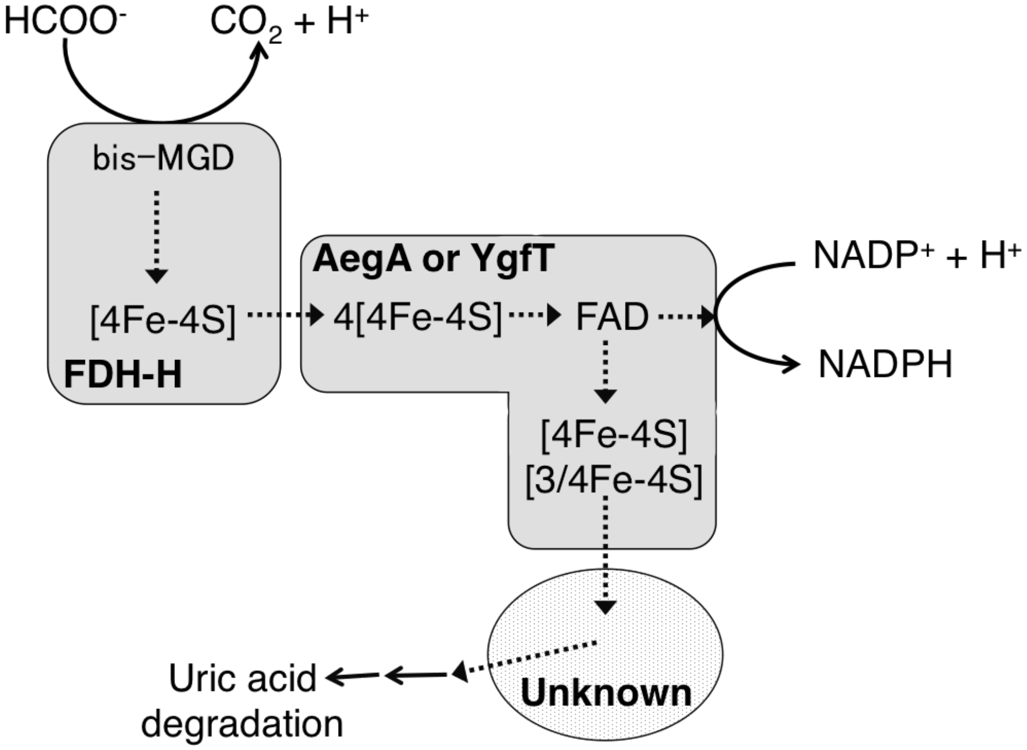

FIG S6 The proposed model of electron flow during FDH-H– and AegA- or YgfT-dependent uric acid degradation. The dashed lines represent the electron flow. First, FDH-H obtains electrons from formate through an essential molybdenum cofactor, bis-molybdopterin guanine dinucleotide (Bis-MGD). Then, the electrons are transferred to the N-terminal ferredoxin-like domains of AegA or YgfT via the iron-sulfur cluster of FDH-H, and subsequently the cofactor FAD of AegA or YgfT is reduced. Finally, $FADH_2$ reduces $NADP^+$ and the other enzyme at the same (bifurcation) or different (bifunction) time.

Table S1. Distribution of enzymes related to AegA/YgfT -dependent uric acid degradation.

Name	AegA P37127.2	YgfT Q46820.2	FDH-H P07658.2	UacT Q46821.2	YgeV Q46802.1	HpxO A6T923.1	HpyO Q8PDQ6.1
Brenneria roseae	PWC19994.1 (62%)	-	PWC18362.1 (67%)	-	-	-	-
Brenneria salicis	WP_113867159.1 (62%)	-	WP_113868509.1 (68%)	-	WP_113866890.1 (59%)	WP_113866833.1 (70%)	-
Citrobacter amalonaticus	WP_103775484.1 (87%)	-	WP_103777760.1 (97%) WP_103775898.1 (65%)	WP_044327227.1 (93%)	WP_042999388.1 (89%)	-	-
Citrobacter braakii	WP_047501608.1 (87%)	-	WP_080756030.1 (95%) WP_047417935.1 (65%)	-	WP_047414829.1 (37%)	-	-
Citrobacter europaeus	WP_087052460.1 (88%)	-	WP_080625853.1 (95%) WP_087050670.1 (65%)	-	-	-	-
Citrobacter farmeri	WP_042317641.1 (88%)	-	WP_084196605.1 (96%) WP_042319512.1 (65%)	WP_042322141.1 (93%)	WP_042322179.1 (88%)	-	-
Citrobacter freundii	WP_003838512.1 (88%)	-	WP_080602221.1 (95%) WP_003030731.1 (65%)	-	WP_003839752.1 (38%)	-	-
Citrobacter koseri	WP_024130130.1 (86%)	-	WP_077956844.1 (97%) ABV14876.1+ABV14879.1 (97%)	-	-	-	-

Citrobacter pasteurii	WP_040233503.1 (88%)	-	WP_082031702.1 (95%) WP_040233894.1 (65%)	-	WP_005122995.1 (38%)	-	-
Citrobacter portucalensis	WP_079935151.1 (88%)	-	WP_079935032.1 (95%) WP_071444730.1 (65%)	-	WP_079934247.1 (38%)	-	-
Citrobacter rodentium	WP_012906603.1 (87%)	WP_012907456.1 (69%)	WP_081442325.1 (97%) WP_081442324.1 (84%)	WP_012907455.1 (42%)	WP_012907467.1 (69%)	-	-
Citrobacter sedlakii	WP_042290898.1 (87%)	-	WP_084589031.1 (97%) WP_042287609.1 (66%)	-	-	-	-
Citrobacter werkmanii	WP_042306888.1 (88%)	-	WP_079225655.1 (95%) WP_042308930.1 (65%)	-	WP_042313222.1 (38%)	-	-
Citrobacter youngae	WP_032939493.1 (88%)	-	WP_084742830.1 (95%) WP_006684908.1 (66%)	-	-	-	-
Dickeya chrysanthemi	WP_027713628.1 (59%)	-	WP_027712528.1 (68%)	-	-	WP_027713194.1 (72%)	-
Dickeya dadantii	WP_013320125.1 (59%)	-	WP_013317224.1 (67%)	-	-	-	-
Dickeya dianthicola	WP_024107897.1 (59%)	-	WP_024105249.1 (67%)	-	-	WP_024104447.1 (73%)	-
Dickeya fangzhongdai	WP_100850366.1 (60%)	-	WP_049854965.1 (65%)	-	-	WP_100848820.1 (72%)	-
Dickeya paradisiaca	WP_015855654.1 (60%)	-	WP_012764887.1 (68%)	-	-	-	-

Dickeya solani	WP_039691991.1 (59%)	-	WP_038660243.1 (67%)	-	-	WP_039692609.1 (72%)	-
Dickeya zeae	WP_019844260.1 (59%)	-	WP_019844833.1 (67%)	-	-	-	-
Edwardsiella hoshinae	WP_024523410.1 (65%)	-	WP_081702264.1 (74%) AOV95968.1 (71%)	WP_024522335.1 (72%)	WP_024523988.1 (73%)	-	-
Edwardsiella ictaluri	WP_015871248.1 (64%)	-	WP_015872499.1 (73%)	WP_015871730.1 (72%)	WP_015872738.1 (72%)	-	-
Edwardsiella piscicida	WP_012848619.1 (65%)	-	WP_078057759.1 (73%) AGH74914.1+AGH74915.1 (82%,70%)	WP_012848998.1 (72%)	WP_015462399.1 (73%)	-	-
Edwardsiella tarda	PVD96283.1 (65%)	-	WP_005296397.1 (73%)	WP_005296993.1 (72%)	WP_005289730.1 (72%)	-	-
Enterobacter cloacae	WP_013098217.1 (78%)	-	WP_013095085.1 +WP_013095086.1 (95%) WP_013096962.1 (65%)	-	-	WP_013097236.1 (81%)	-
Erwinia mallotivora	-	WP_034934316.1 (63%)	WP_034934312.1 (62%)	-	WP_084276176.1 (54%)	WP_034935945.1 (69%)	-
Erwinia oleae	-	WP_034945038.1 (64%)	WP_034945033.1 (61%)	WP_052118369.1 (43%)	WP_034945233.1 (51%)	WP_034950873.1 (27%)	-
Erwinia teleogrylli	WP_058913314.1 (74%)	-	WP_082683201.1 (87%)	-	WP_058910632.1 (38%)	-	-

Escherichia coli	P37127.2 (100%)	Q46820.2 (100%)	P07658.2 (100%)	Q46821.2 (100%)	Q46802.1 (100%	-	-
Hafnia alvei	WP_004089824.1 (65%)	-	WP_087944325.1 (75%) WP_004093289.1 (68%)	WP_004095720.1 (70%)	WP_035504355.1 (72%)	-	-
Hafnia paralvei	WP_008813462.1 (65%)	-	WP_082019157.1 (75%) WP_082919470.1 (68%)	WP_039188795.1 (70%)	WP_008815430.1 (72%)	-	-
Klebsiella aerogenes	WP_108684119.1 (77%)	-	WP_108683964.1 (94%) WP_108684333.1 (92%)	WP_015366914.1 (41%)	WP_015366900.1 (75%)	WP_045361186.1 (80%)	-
Klebsiella grimontii	WP_098141014.1 (79%)	WP_098140839.1 (67%)	WP_098140218.1 (95%) WP_077253451.1 (86%)	WP_004133613.1 (43%)	SNU36138.1 (73%)	WP_004871444.1 (84%)	-
Klebsiella oxytoca	WP_014230339.1 (78%)	WP_014229769.1 (67%)	WP_077598877.1 (86%)	WP_014229771.1 (43%)	AEX06366.1 (73%)	WP_014228756.1 (84%)	-
Klebsiella pneumoniae	WP_008803840.1 (76%)	-	WP_012543160.1 (95%) WP_012541439.1 (66%)	-	-	WP_008804924.1 (99%)	-
Klebsiella quasipneumoniae	WP_109547067.1 (76%)	-	WP_048335933.1 (66%) WP_077598907.1 (95%)	-	-	WP_023289663.1 (99%)	-
Klebsiella variicola	WP_012967460.1 (76%)	-	WP_012969145.1 (94%) WP_012967957.1 (66%)	-	-	WP_012968247.1 (98%)	-

Kosakonia arachidis	WP_090124753.1 (80%)	-	WP_090121893.1 (94%) SFT92868.1+SFT92859.1 (95%, 94%) WP_090120916.1 (67%) WP_090125349.1 (33%)	-	-	WP_090119467.1 (83%)	-
Kosakonia cowanii	APZ07517.1 (78%)	-	APZ04247.1 +APZ04248.1 (94%, 92%) APZ04494.1 (66%) APZ06820.1 (35%)	-	APZ07402.1 (71%)	-	-
Kosakonia oryzae	WP_064564598.1 (82%)	-	WP_064564176.1 (66%) WP_082934132.1 (94%)	-	-	WP_064567402.1 (83%)	-
Kosakonia oryzendophytica	WP_088238185.1 (81%)	-	WP_082794188.1 (95%) SCC47526.1+SCB85072.1 (96% , 94%) WP_061493444.1 (67%) WP_088237880.1 (34%)	-	-	-	-
Kosakonia oryziphila	WP_090136254.1 (81%)	-	WP_090136680.1 (95%) SCC47511.1 (94%) WP_090134534.1 (66%) WP_090134032.1 (34%)	-	-	WP_090136419.1 (82%)	-

Kosakonia pseudosacchari	WP_097400306.1 (82%)	-	WP_086873959.1 (95%) WP_086873690.1 (67%) WP_097399640.1 (35%)	-	-	-	-
Kosakonia radicincitans	WP_007370887.1 (81%)	-	WP_007370533.1 (66%) WP_007371777.1 (33%) WP_079517076.1 (95%)	-	-	WP_007374278.1 (83%)	WP_007372550.1 (37%)
Kosakonia sacchari	WP_065370391.1 (82%)	-	WP_065368325.1 (34%) WP_083126964.1 (95%) WP_017459296.1 (67%)	-	-	-	-
Morganella morganii	-	WP_062772669.1 (64%)	WP_062772369.1 (64%)	WP_062773079.1 (32%)	WP_049246430.1 (64%)	-	-
Morganella psychrotolerans	-	WP_067404336.1 (64%)	WP_067404760.1 (65%)	WP_067404325.1 (77%)	WP_067424175.1 (63%)	-	-
Pectobacterium atrosepticum	WP_011095917.1 (61%)	-	WP_011093088.1 (66%) WP_011092840.1 (64%)	-	-	-	-
Pectobacterium betavasculorum	WP_039307596.1 (61%)	-	WP_039307451.1 (66%) WP_039301898.1 (65%)	-	-	WP_039303411.1 (73%)	-
Pectobacterium carotovorum	PXB00685.1 (61%)	-	PXB03217.1 (66%)	-	-	-	-
Pectobacterium parmentieri	WP_015731593.1 (61%)	-	WP_015730221.1 (66%)	-	-	-	-

Pectobacterium peruviense	WP_113627154.1 (62%)	-	WP_113625582.1 (66%) WP_113627286.1 (65%)	-	-	-	-
Pectobacterium polaris	WP_109412130.1 (61%)	-	WP_109412017.1 (65%)	-	-	-	-
Pectobacterium wasabiae	WP_005976634.1 (61%)	-	WP_005970873.1 (66%)	-	-	-	-
Rahnella aquatilis	AFE58521.1 (57%)	-	WP_015689505.1 (68%)	-	-	WP_015690544.1 (68%)	-
Raoultella ornithinolytica	WP_004866135.1 (76%)	WP_004862850.1 (69%)	WP_004863070.1 (65%)	WP_004862857.1 (84%)	WP_004862816.1 (75%)	WP_004860305.1 (81%)	-
Raoultella planticola	WP_032699036.1 (77%)	WP_032699623.1 (68%)	WP_080718653.1 (93%) WP_032699577.1 (65%)	WP_050500315.1 (84%)	WP_032696016.1 (76%)	WP_032700349.1 (84%)	-
Raoultella terrigena	WP_045856230.1 (77%)	WP_045858654.1 (69%)	WP_045858779.1 (66%)	WP_052698750.1 (84%)	WP_045858635.1 (75%)	WP_045854751.1 (81%)	-
Salmonella bongori	AGR59776.1 (84%)	-	AGR61457.1 (98%)	-	-	-	-
Salmonella enterica	EGE30645.1 (84%)	-	EGE32322.1 (98%)	-	-	-	-
Serratia fonticola	WP_021806731.1 (58%)	-	WP_021806458.1 (73%)	ERK10812.1 (71%)	ERK14431.1 (69%)	-	-
Serratia oryzae	WP_083704380.1 (58%)	WP_076941014.1 (61%)	WP_083704367.1 (89%) WP_076941254.1 (65%)	WP_076941013.1 (82%)	WP_076942367.1 (67%)	-	-
Serratia rubidaea	WP_061325023.1 (60%)	-	WP_061325026.1 (65%)	AML60097.1 (44%)	AML60107.1 (62%)	WP_061324419.1 (82%)	-
Shigella boydii	EFW55379.1 (99%)	EFW57118.1 (99%)	WP_001300547.1 (100%)	WP_024183589.1 (99%)	-	-	-

Shigella dysenteriae	EFW51348.1 (99%)	-	EFW49573.1 (99%)	EFW51004.1 (99%)	-	-	-
Shigella flexneri	EGJ85869.1 (99%)	-	EGJ82573.1 +EGJ82575.1 (99%)	EGJ84101.1 (99%)	-	-	-
Shigella sonnei	EIQ52090.1 (99%)	EIQ51256.1 (99%)	EIQ49720.1 + EIQ49730.1 (99%, 100%)	EIQ51239.1 (99%)	EIQ51238.1 (99%)	-	-
Shimwellia blattae	WP_002441264.1 (72%)	-	WP_002441512.1 (92%) AFJ48682.1 (90%) WP_002442990.1 (51%)	-	-	-	-
Yersinia aleksiciae	WP_048618975.1 (56%)	-	WP_048618975.1 (56%)	-	-	-	-
Yersinia bercovieri	WP_005275836.1 (55%)	-	WP_005273825.1 (66%)	-	-	-	-
Yersinia enterocolitica	WP_050142752.1 (56%)	-	WP_050142756.1 (67%)	-	-	-	-
Yersinia frederiksenii	WP_038636999.1 (56%)	-	WP_038634346.1 (67%) WP_038636995.1 (63%)	-	-	-	-
Yersinia intermedia	WP_005187388.1 (57%)	-	EEQ18742.1 (67%) EEQ18873.1 +EEQ18874.1 (62%)	-	-	-	-
Yersinia kristensenii	WP_050124898.1 (56%)	-	WP_050127144.1 (66%)	-	-	-	-

Yersinia mollaretii	WP_004873957.1 (56%)	-	WP_004876281.1 (67%) WP_032813278.1 (63%)	-	-	-	-
Yersinia pestis	WP_002209118.1 (56%)	-	WP_002209120.1 (63%)	-	-	-	-
Yersinia pseudotuberculosis	WP_071990600.1 (57%)	-	WP_011191608.1 (63%)	-	-	-	-
Yersinia similis	WP_054878385.1 (55%)	-	WP_025382348.1 (63%)	-	-	-	-
Yersinia wautersii	WP_033848955.1 (56%)	-	WP_033848953.1 (63%)	-	-	-	-

TABLE S2 Strains and plasmids used in the current study

Strain or plasmid	Genotype or source
E. coli strain	
aegA'-lacZ	ins:Cm^{r}~[*aegA'-lacZ*$^{+}$]
ygfT'-lacZ	ins:Cm^{r}~[*ygfT'-lacZ*$^{+}$]
MG1655	wild-type K-12 MG1655
Δ*aegA*	Δ*aegA*::Sm^{r}
Δ*ygfTS*	Δ*ygfTS*::Cm^{r}
Δ*aegA*Δ*ygfTS*	Δ*aegA*::Sm^{r} Δ*ygfTS*::Cm^{r}
MG1655 mFKm	MG1655 mFKm
Δ*aegA*Δ*ygfTS* mFKm *aegA*	Δ*aegA*::Sm^{r} Δ*ygfTS*::Cm^{r} mFKm *aegA*
ΔaegAΔygfTS mFKm *ygfT*	Δ*aegA*::Sm^{r} Δ*ygfTS*::Cm^{r} mFKm *ygfTS*
Δ*hydN*Δ*ygfS*	Δ*hydN*::Tc^{r} Δ*ygfS*::Cm^{r}
Δ*hydN*Δ*ygfS*Δ*aegA*	Δ*hydN*::Tc^{r} Δ*ygfS*::Cm^{r} Δ*aegA*::Sm^{r}
Δ*hydN*Δ*ygfTS*	Δ*hydN*::Tc^{r} Δ*ygfTS*::Cm^{r}
Δ*hydN*Δ*ygfTS*Δ*aegA*	Δ*hydN*::Tc^{r} Δ*ygfTS*::Cm^{r} Δ*aegA*::Sm^{r}
ΔHYD	Δ*hyaA-C*::Ap^{r} Δ*hybC-O*::Gen^{r} Δ*hycB-G*::Tc^{r} Δ*hyfA-I*::Ap^{r}
ΔHYDΔ*aegA*	Δ*hyaA-C*::Ap^{r} Δ*hybC-O*::Gen^{r} Δ*hycB-G*::Tc^{r} Δ*hyfA-I*::Ap^{r} Δ*aegA*:Sm^{r}
ΔHYDΔ*ygfTS*	Δ*hyaA-C*::Ap^{r} Δ*hybC-O*::Gen^{r} Δ*hycB-G*::Tc^{r} Δ*hyfA-I*::Ap^{r} Δ*ygfTS*::Cm^{r}
ΔHYDΔ*aegA*Δ*ygfTS*	Δ*hyaA-C*::Ap^{r} Δ*hybC-O*::Gen^{r} Δ*hycB-G*::Tc^{r} Δ*hyfA-I*::Ap^{r} Δ*aegA*::Sm^{r} Δ*ygfTS*::Cm^{r}
ΔFDH	Δ*fdhF*::Ap^{r} Δ*fdnGHI*::Km^{r} Δ*fdoGHI*::Tc^{r}
ΔFDHΔ*aegA*	Δ*fdhF*::Ap^{r} Δ*fdnGHI*::Km^{r} Δ*fdoGHI*::Tc^{r} Δ*aegA*::Sm^{r}
ΔFDHΔ*ygfTS*	Δ*fdhF*::Ap^{r} Δ*fdnGHI*::Km^{r} Δ*fdoGHI*::Tc^{r} Δ*ygfTS*:Cm^{r}
ΔFDHΔ*aegA*Δ*ygfTS*	Δ*fdhF*::Ap^{r} Δ*fdnGHI*::Km^{r} Δ*fdoGHI*::Tc^{r} Δ*aegA*::Sm^{r} Δ*ygfTS*:Cm^{r}
Δ*fdhD*	Δ*fdhD*::Tc^{r}
ΔFDH-H	Δ*fdhF*::Ap^{r}
ΔFDH-N	Δ*fdnGHI*::Km^{r}
ΔFDH-O	Δ*fdoGHI*::Tc^{r}
ΔFDH-HΔFDH-N	Δ*fdhF*::Ap^{r} Δ*fdnGHI*::Km^{r}
ΔFDH-NΔFDH-O	Δ*fdnGHI*::Km^{r} Δ*fdoGHI*::Tc^{r}
ΔFDH-OΔFDH-H	Δ*fdoGHI*::Tc^{r} Δ*fdhF*::Ap^{r}
ΔFDH-HΔFDH-NΔFDH-O	Δ*fdhF*::Ap^{r} Δ*fdnGHI*::Km^{r} Δ*fdoGHI*::Tc^{r}
MG1655 mFTc	MG1655 mFTc
ΔFDH-H mFTc	Δ*fdhF*::Ap^{r} mFTc
ΔFDH-H mFTc FDH-H	Δ*fdhF*::Ap^{r} mFTc FDH-H
ΔFDH-HΔ*aegA*Δ*ygfTS*	Δ*fdhF*::Ap^{r} Δ*aegA*::Sm^{r} Δ*ygfTS*::Cm^{r}
Δ*pflAB*	Δ*pflAB*::Tc^{r}
Δ*pflAB*ΔFDH-H	Δ*pflAB*::Tc^{r} Δ*fdhF*::Ap^{r}
Δ*pflAB*Δ*aegA*Δ*ygfTS*	Δ*pflAB*::Tc^{r} Δ*aegA*::Sm^{r} Δ*ygfTS*::Cm^{r}
Δ*pflAB*ΔFDH-HΔ*aegA*Δ*ygfTS*	Δ*pflAB*::Tc^{r} Δ*fdhF*::Ap^{r} Δ*aegA*::Sm^{r} Δ*ygfTS*::Cm^{r}
OCL42	(2)
OCL43	(2)
Δ*ygeV*	Δ*ygeV*::Cm^{r}
OCL42Δ*pflAB*	OCL42 Δ*pflAB*::Tc^{r}
OCL43Δ*pflAB*	OCL43 Δ*pflAB*::Tc^{r}
OCL43 *ygfT'-lacZ*	OCL43 ins:Cm^{r}~[*ygfT'-lacZ*$^{+}$]
Δ*ygeV* *ygfT'-lacZ*	Δ*ygeV*::Ap^{r} ins:Cm^{r}~[*ygfT'-lacZ*$^{+}$]
Δ21a	(3)
Δ21aΔ*aegA*	Δ21a Δ*aegA*::Cm^{r}

Δ21aΔ*aegA* mFKm	Δ21a Δ*aegA*::Cm^{r} mFKm
Δ21aΔ*aegA* mFKm *aegA*	Δ21a Δ*aegA*::Cm^{r} mFKm *aegA*
Δ*ygfT aegA*(Wt)	Δ*ygfT*::Km^{r} ins:*aegA*(Wt)~Cm^{r}
Δ*ygfT aegA*(C68A)	Δ*ygfT*::Km^{r} ins:*aegA*(C68A)~Cm^{r}
Δ*ygfT aegA*(C97A)	Δ*ygfT*::Km^{r} ins:*aegA*(C97A)~Cm^{r}
Δ*ygfT aegA*(C121A)	Δ*ygfT*::Km^{r} ins:*aegA*(C121A)~Cm^{r}
Δ*ygfT aegA*(C230A)	Δ*ygfT*::Km^{r} ins:*aegA*(C230A)~Cm^{r}
Δ*ygfT aegA*(G334A G336A)	Δ*ygfT*::Km^{r} ins:*aegA*(G334A G336)~Cm^{r}
Δ*ygfT aegA*(G447A)	Δ*ygfT*::Km^{r} ins:*aegA*(G447A)~Cm^{r}
Δ*ygfT* His-AegA	Δ*ygfT*::Km^{r} ins:His-*aegA*~Cm^{r}
Δ*ygfT* AegA-His	Δ*ygfT*::Km^{r} ins:*aegA*-His~Cm^{r}
Δ*ygfT*Δ*aegA*	Δ*ygfT*::Km^{r} Δ*aegA*::Cm^{r}
Plasmid	
mFKm	Km^{r}
mFKm *aegA*	mFKm containing *aegA*
mFKm *ygfTS*	mFKm containing *ygfTS*
mFTc	Tc^{r}
mFTc FDH-H	mFTc containing *fdhF*

TABLE S3 Primers used in the current study

Primer	Sequence (5′–3′)
142-3	CCTCACGACTCGGACAAAATGTCGTTGCGC
Cm N	CTGGTGTCCCTGTTGATACC
Cm C	CACTTATTCAGGCGTAGCAC
141-24	ATGACCATGATTACGGATTC
141-25	TTCAACCACCGCACGATAGAG
141-22	CGATCGGCATAACCACCACG
141-21	ATCAAAACATGCCCGAATGTGCACC
141-18	AAACTGTTACCCGTAGGTAGTCACG
333-22	TTGTAAAACGACGGCCAGTGAATCCGTAATCATGGTCATAACGACCTTCTTTTTGGTTG
333-23	AAGTTGGCCCAGGGCTTCCCGGTATCAACAGGGACACCAGGCAGCATTTTGCGTTCAGCG
372-15-2	GGTATCAACAGGGACACCAGCACCGGCAGGCGGATCCCCA
372-16	GAATCCGTAATCATGGTCATTTGCATCCCTTTCATTTAATGAG
aegA-CmN	AAGGTTTTTACAACCAAAAAAGAAGGTCGTTATGAATCGT CTGGTGTCCCTGTTGATACC
aegA-CmC	CGCTGTGAAGTCGGGTTTGTCTGCGCAGGCTATCAGTGAG CACTTATTCAGGCGTAGCAC
CmN'-SaApL	TCCCAATGGCATCGTAAAGAACATTTTGAGGCATTTCAGTAATAACTTAGCCATTTCAACACC
CmC'-SaApR	TGAAAACGGGGGCGAAGAAGTTGTCCATATTGGCCACGTTATAAATCTCGAAAATAATAGAGGG
ygfT-CmN	AGACATAACTCATTAAATGAAAGGGATGCAAATGAATAAGCTGGTGTCCCTGTTGATACC
ygfS-CmC	AGATTCTCCGCGCCTTTCAGCGCGGAGATTTCTTTCAGAGCACTTATTCAGGCGTAGCAC
333-42	GAGTCAATTCAGGGTGGTGAATGTGAAACCAGTAACGTTAGTGGAAAGAATCGACAGCAG
333-46	CTTGTGCAATGTAACATCAGAGATTTTGAGACACAACGTG GAGGGTGATTTGTTGCGTCA
333-45	ACGCTCAGTGGAACGAAAACTCACGTTAAGGGATTCAATCACACGCGTCTTCTCC
372-18	CTTGTGCAATGTAACATCAGAGATTTTGAGACACAACGTG GATTCTCCGCGCCTTTCAGC
372-19	CCGAATTC GCTTTCCTGGTGATAATATGCGGTC
372-17	ACGCTCAGTGGAACGAAAACTCACGTTAAGGGATTGTCTAAACACATGCTCGTGG
CmN-KmC	CTGGTGTCCCTGTTGATACCGGGAAGCCCTGGGCCAACTTC AAGTCAGCGTAATGCTCTG
hydN-CmN	CGGCGCATTAACTGTCATTGCTGGAGAATTTGATGAACCGCTGGTGTCCCTGTTGATACC
hydN-CmC	CTTTGATGCCAGAACAGTTCTGTAAGATTTTTAGAACATCCACTTATTCAGGCGTAGCAC
ygfS-CmN	TGTTAACTCTGTTTGATACGAAGGCATCGTGATGAAATCGCTGGTGTCCCTGTTGATACC
hyaA-SaApL	GTGTCGCAAGGAGGAGAGACGTGCGATATGAATAACGAATAACTTAGCCATTTCAACACC
hyaC-SaApR	GCCCCATGACCACCACGCGTTGCTCGCTCATGAACGATAAATCTCGAAAATAATAGAGGG
hybO-SaApL	CAGGTCTTCGCAACGGAATAACTATAAATGACTGGAGAATAACTTAGCCATTTCAACACC
hybC-SaApR	TGCCGACCCCTAAGACTAAAATACGCATTACAGAACATAAATCTCGAAAATAATAGAGGG
hycG-CmC	CGGCTCAGTTGACTGAACACCACCTTTTCACTCATCGGATCACTTATTCAGGCGTAGCAC

hycB-CmN	TCAAAAATGACAATCACCTGAGGAATGCCTGGTGAATCGTCTGGTGTCCCTGTTGATACC
hyfA-SaApL	TGTTTCATATCATTTTCAAGGAGCCGACATGAACCGCAATAACTTAGCCATTTCAACACC
hyfI-SaApR	GTCCAGAAAACAATTTCCCCGCACTCTTCAGTCATGATAAATCTCGAAAATAATAGAGGG
fdhF-TcN	TGCGTGATTTGATTAACTGGAGCGAGACCGATGAAAAAAGCCGACCTCATTAAGCAGCTC
fdhF-TcC	CCTCCGAAAGGAGGCTGTAGAAAGGACGGTATTACGCCAGGGCTGGTTTATGCATATCGC
fdhE-TcC	GGCGACCTGCGCCACCCGGAAAACGTCAGAGATTACTCTCGGCTGGTTTATGCATATCGC
fdoG-TcN	GACGTGACAATGTCGAAACAAGGAGCAATCCATGCAGGTCCCGACCTCATTAAGCAGCTC
fdnG-CmN	GCAGTAATACCCCTGAAAAAAGAGGAAAGCAATGGACGTCCTGGTGTCCCTGTTGATACC
fdnI-CmC	GCTTAAAGTTCAAAAGAGATTATATCCCTTCTTCACTCTCCACTTATTCAGGCGTAGCAC
fdhD-TcN	GACTTATTTTAATTAATTGCTCAAACGCTGCGGATGGGTACCGACCTCATTAAGCAGCTC
fdhD-TcC	CATAATGTTGGTGTGTGTGTTCTTATCTGGTTAAGAGAAAGGCTGGTTTATGCATATCGC
TcN	CCGACCTCATTAAGCAGCTC
TcC	GGCTGGTTTATGCATATCGC
481-7-2	TTGCTTGTGGATAGTCCAGACTGAC
481-8	TTTTTCGCATGTATTGCGATATGCATAAACCAGCCACAAACTCACGATGTGATTGATTAC
pflA-CmN	ATGTTCTAATTTCCCCGTAAAGCGGCAACTTTATTGAGTTCTGGTGTCCCTGTTGATACC
pflB-CmC	CTCGGACATGTAACACCTACCTTCTTAAGTGGATTTTTTACACTTATTCAGGCGTAGCAC
CmN-TcN	CTGGTGTCCCTGTTGATACCGGGAAGCCCTGGGCCAACTTCCGACCTCATTAAGCAGCTC
CmC-TcC	CACTTATTCAGGCGTAGCACCAGGCGTTTAAGGGCACCAAGGCTGGTTTATGCATATCGC
ygeVN-CmN	GACTTTTAAGTAAGTCTGGAAGGTAAAGCTATGGAGCTTGCTGGTGTCCCTGTTGATACC
ygeVC-CmC	ATCCGGCCTGAATTCAGGCCGGATTCACTGAGGTTATGTGCACTTATTCAGGCGTAGCAC
ygfT-CmC	GTCAGCCGGATTAACGATAATTAACGATTTCATCACGATGCACTTATTCAGGCGTAGCAC
333-28-2	TTGCGCCATTAGGGGCGCTACGGGCGCAGGGCGCATCTTC
333-29	CCCTGCGCCCGTAGCGCCCCTAATGGCGCAATCAGCCACGTTG
333-30	TGCATCGTACCAAAAGGCGCGGCCACCACGCAGGATTTAC
333-31	ATCCTGCGTGGTGGCCGCGCCTTTTGGTACGATGCAAATC
333-32	CCCCGCACAAAGGTCCGCTTTATGCGCCGTGGCTTTTACTTTTCC
333-33	AGTAAAAGCCACGGCGCATAAAGCGGACCTTTGTGCGGGGCGCGA
333-34	AAACGCTGTGCTCGCCCGCCTTAAGGCAGCGCGAGGCTTC
333-35	CGCGCTGCCTTAAGGCGGGCGAGCACAGCGTTTGTGAATG
333-36	GCCAGCCCTGCCGGGGCTGCGGCGATAATCGCCACCCGCTTGT
333-37	TGGCGATTATCGCCGCAGCCCCGGCAGGGCTGGCCTGTGC
333-38	TCCATCGCGGTGTCGGCGCCGCCCAGTACCACGACGTTAAG
333-39	GTCGTGGTACTGGGCGGCGCCGACACCGCGATGGACTGTGT
333-40	TGGCCCAGGGCTTCCCGGTATCAACAGGGACACCAGTCAGTGAGATTTGACTGATTTTAC
333-41	GTAAACCGTCTCTTGCATTG
333-8	GGCTCAGGGTCATCACCAGTCCATT
333-11	CTCAATACGTAAAACGGCATCGGTA

333-16	GGAAACAATGCACATCAATCTGGCT
333-5	CCGGATCCAATCACACGCGTCTTCTCC
333-7	GCCATGTCACCAAAGTGGACAAGCG
333-8	GGCTCAGGGTCATCACCAGTCCATT
333-9	TTCGAATTCCCATATGGTACCAGCTGCAGATCTCGGTGAGATTTGACTGATTTTACCCCC
333-10	GCCCTTAAACGCCTGGTGCTACGCCTGAATAAGTGTAGCCTGCGCAGACAAACCCGACTT
pBAD-N (TI)	CGAGATCTGCAGCTGGTACC
pBAD-N5	CCCTCGAGATCTGCAGCTGGTACCATATGGG
pBAD-HisC	GCTAGCCATACCATGATGATGATGA
333-17	GTTCTCATCATCATCATCATCATGGTATGGCTAGCATGAATCGTTTTATTATGGCCAACA
333-13	CCCTGCAGTCAGTGAGATTTGACTGATT
333-15	ATACCATGATGATGATGATGATGAGAACCCCCCATAACGACCTTCTTTTTTGGTTGTAAA
333-14	GGCCCAGGGCTTCCCGGTATCAACAGGGACACCAGTCAGTGAGATTTGACTGATTTTACC
pBAD-HisN	ATGGGGGGTTCTCATCATCATCATC
334-15	CCAAGCTTTGGTTGGGATGTTGTGCGACAAAAC
333-16	GGAAACAATGCACATCAATCTGGCT

TABLE S4 Primer-template sets used in the current study

No.	Product	Primer 1	Primer 2	Template 1	Template 2	Template 3
1	lacIN	142-3	141-78	Chromosome	-	-
2	Cm	Cm N	Cm C	pACYC184	-	-
3	LacZ	141-24	141-25	Chromosome	-	-
4	PaegA	333-23	333-22	Chromosome	-	-
5	PygfT	372-15-2	372-16	Chromosome	-	-
6	LacIN-Cm	141-23	Cm N	lacIN	Cm	-
7	Cm-PaegA	Cm C	333-22	Cm	PaegA	-
8	Cm-PygfT	Cm C	372-16	Cm	PygfT	-
9	PaegA-lacZ	333-23	141-22	PaegA	LacZ	-
10	PygfT-lacZ	372-15-2	141-22	PygfT	LacZ	-
11	**lacIN-Cm-PaegA-LacZ**	141-21	141-18	LacIN-Cm	Cm-PaegA	PaegA-lacZ
12	**lacIN-Cm-PygfT-LacZ**	141-21	141-18	LacIN-Cm	Cm-PygfT	PygfT-lacZ
13	Cm:Sm	CmN'-SaApL	CmC'-SaApR	Ap:Sm	-	-
14	**aegA:Sm**	aegA-CmN	aegA-CmC	Cm:Sm	-	-
15	**ygfTS:Cm**	ygfT-CmN	ygfS-CmC	pACYC184	-	-
16	aegA	333-42	333-46	Chromosome	-	-
17	ygfTS	372-18	372-19	Chromosome	-	-
18	Km-aegA	KmN	333-45	aegA	Km	-
19	Km-ygfTS	KmN	372-17	ygfTS	Km	-
20	**hydN:Tc**	hydN-CmN	hydN-CmC	Cm:Tc	-	-
21	**ygfS:Cm**	ygfS-CmC	ygfS-CmN	pACYC184	-	-
22	**fdhF:Ap**	fdhF-TcN	fdhF-TcC	Tc:Ap	-	-
23	**fdoGHI:Tc**	fdhE-TcC	fdoG-TcN	Tc	-	-
24	**fdnGHI:Km**	fdnG-CmN	fdnI-CmC	Cm:Km	-	-
25	**fdhD:Tc**	fdhD-TcN	fdhD-TcC	Tc	-	-
26	Tc	TcN	TcC	-	-	-
27	fdhF	481-7-2	481-8	Chromosome	-	-
28	Tc-fdhF	TcN	481-5	Tc	fdhF	
29	**pflAB:Tc**	pflA-CmN	pflB-CmC	Cm:Tc	-	-
30	**ygeV:Cm**	ygeVN-CmN	ygeVC-CmC	pACYC184	-	-
31	**ygfT:Km**	ygfT-CmN	ygfT-CmC	Cm:Km	-	-
32	aegAL	333-5	333-9	Chromosome	-	-
33	MycHis+Cm	pBADN	CmC	pBAD/MycHisA CmF	-	-
34	aegAR	333-10	333-11	Chromosome	-	-
35	aegAL-MycHis+Cm	333-5	CmC	aegAL	MycHis+Cm	-
36	MycHis+Cm-aegAR	pBADN	333-11	MycHis+Cm	aegAR	-
37	**aegAL-MycHis+Cm-aegAR**	333-7	333-8	aegAL-MycHis+Cm	MycHis+Cm-aegAR	-

38	Cm+aegAR	CmN	333-11	aegAL-MycHis+Cm-aegAR	-	-
39	RaegA (C68A)	333-40	333-29	Chromosome	-	-
40	LaegA (C68A)	333-41	333-28-2	Chromosome	-	-
41	**aegA (C68A)+Cm**	333-41	333-11	Cm+aegAR	RaegA (C68A)	LaegA (C68A)
42	RaegA (C97A)	333-40	333-31	Chromosome	-	-
43	LaegA (C97A)	333-41	333-30	Chromosome	-	-
44	**aegA (C97A)+Cm**	333-41	333-11	Cm+aegAR	RaegA (C97A)	LaegA (C97A)
45	RaegA (C121A)	333-40	333-33	Chromosome	-	-
46	LaegA (C121A)	333-41	333-32	Chromosome	-	-
47	**aegA (C121A)+Cm**	333-41	333-11	Cm+aegAR	RaegA (C121A)	LaegA (C121A)
48	RaegA (C230A)	333-40	333-35	Chromosome	-	-
49	LaegA (C230A)	333-41	333-34	Chromosome	-	-
50	**aegA (C230A)+Cm**	333-41	333-11	Cm+aegAR	RaegA (C230A)	LaegA (C230A)
51	RaegA(G334A G336A)	333-40	333-37	Chromosome	-	-
52	LaegA(G334A G336A)	333-41	333-36	Chromosome	-	-
53	**aegA(G334A G336A)+Cm**	333-41	333-11	Cm+aegAR	RaegA (G334A G336A)	LaegA(G334A G336A)
54	RaegA (G447A)	333-40	333-39	Chromosome	-	-
55	LaegA (G447A)	333-41	333-38	Chromosome	-	-
56	**aegA(G447A)+Cm**	333-41	333-11	Cm+aegAR	RaegA (G447A)	LaegA (G447A)
57	L-aegA	334-15	333-15	Chromosome	-	-
58	pBAD-His	pBAD-N5	pBAD-HisC	pBAD / His	-	-
59	aegA	333-17	333-8	Chromosome	-	-
60	pBAD-His-aegA	pBAD-HisN	333-14	pBAD-His	aegA	-
61	**His-aegA+Cm**	333-16	333-8	L-aegA	pBAD-His-aegA	Cm+aegAR
62	Km	CmN-KmC	KmN	Km	-	-

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