Supplementary Material

Genetic architecture and fitness of bacterial inter-species hybrids

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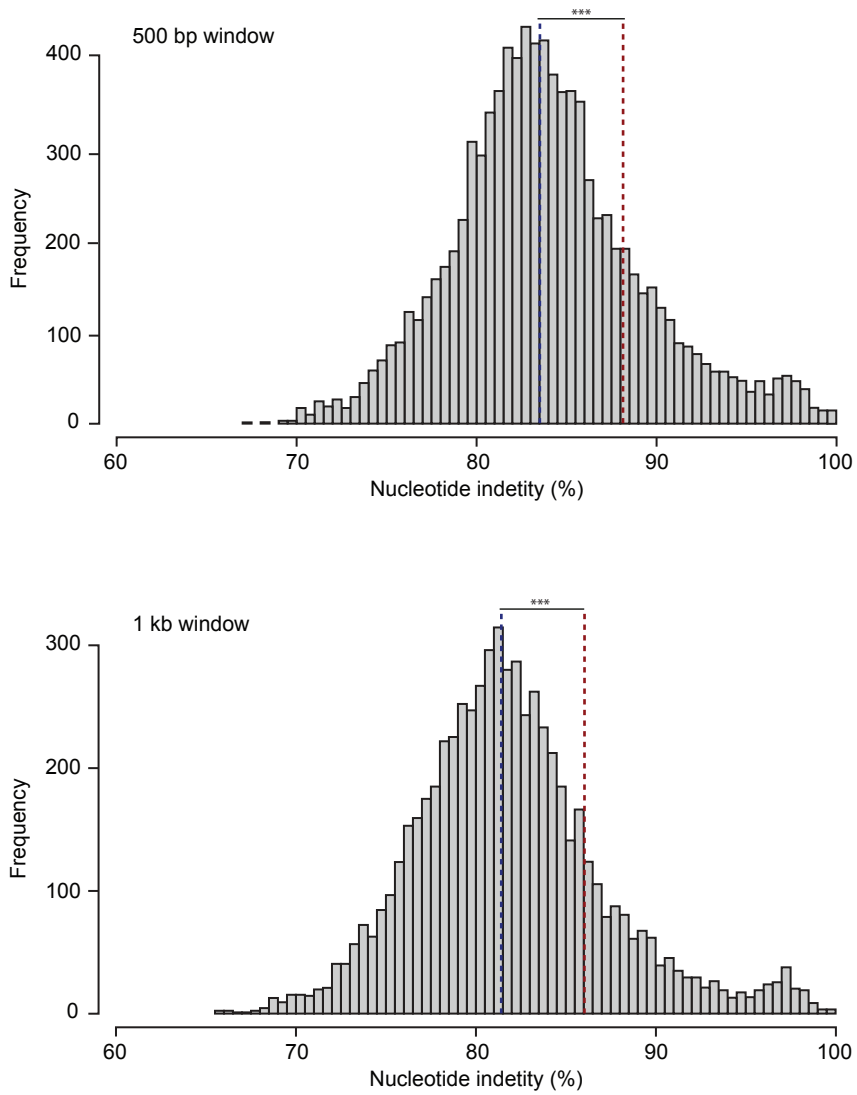
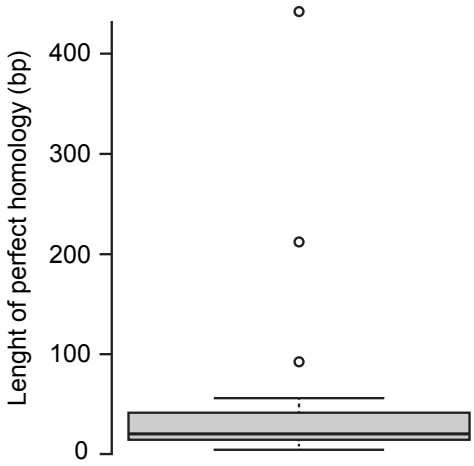
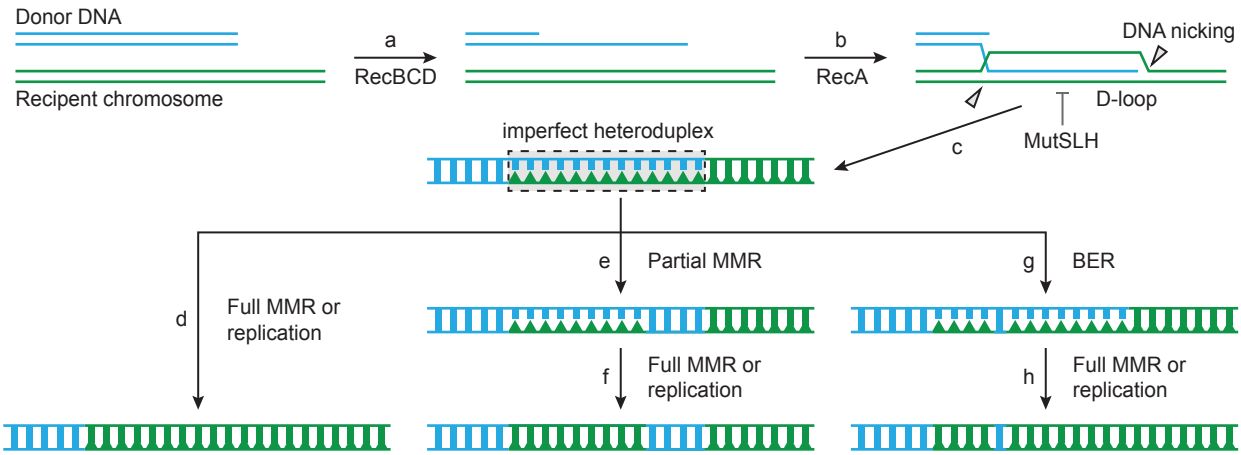
Key words: Conjugation, experimental evolution, *Escherichia coli*, *Salmonella* Typhimurium, relative fitness, recombination



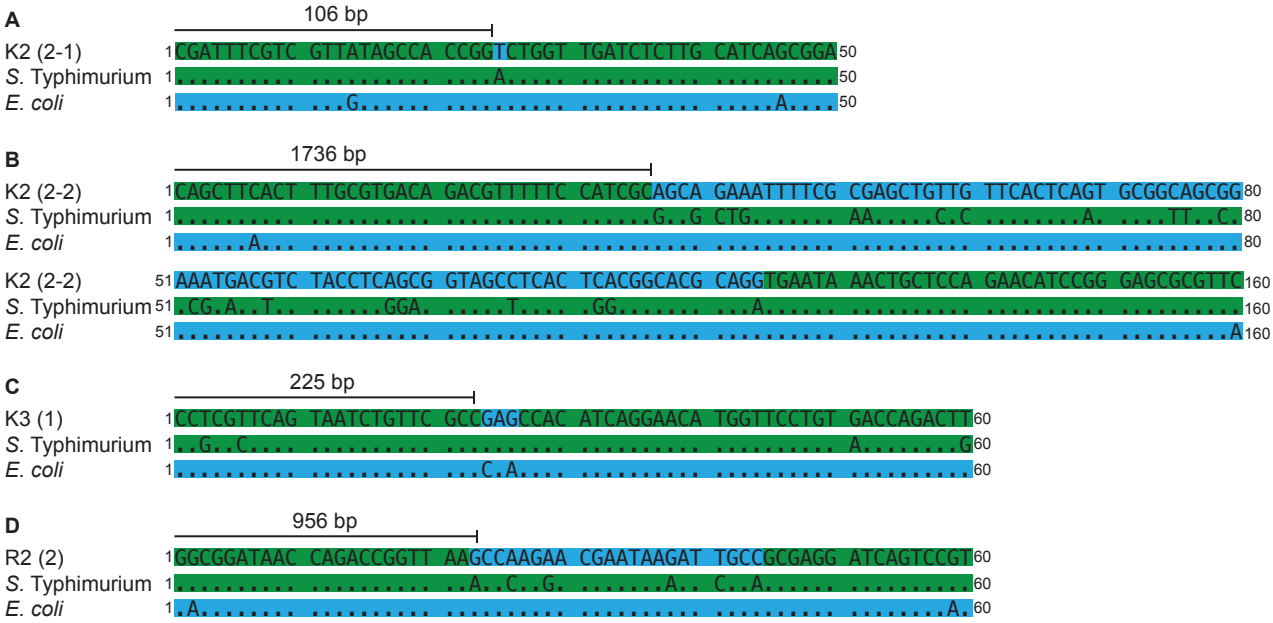
Supplementary FIG S1. Comparison of *E. coli* and *S.* Typhimurium nucleotide identity. Average nucleotide identity between the chromosomes of the *E. coli* donor and *S.* Typhimurium recipient with a sliding 500 bp (top) and 1 kb (bottom) window. Dotted lines indicate average nucleotide identity of the two chromosomes (blue) and the recombination junctions (red). ***P-value <0.001 (two-tailed Kolmogorov-Smirnov Test).



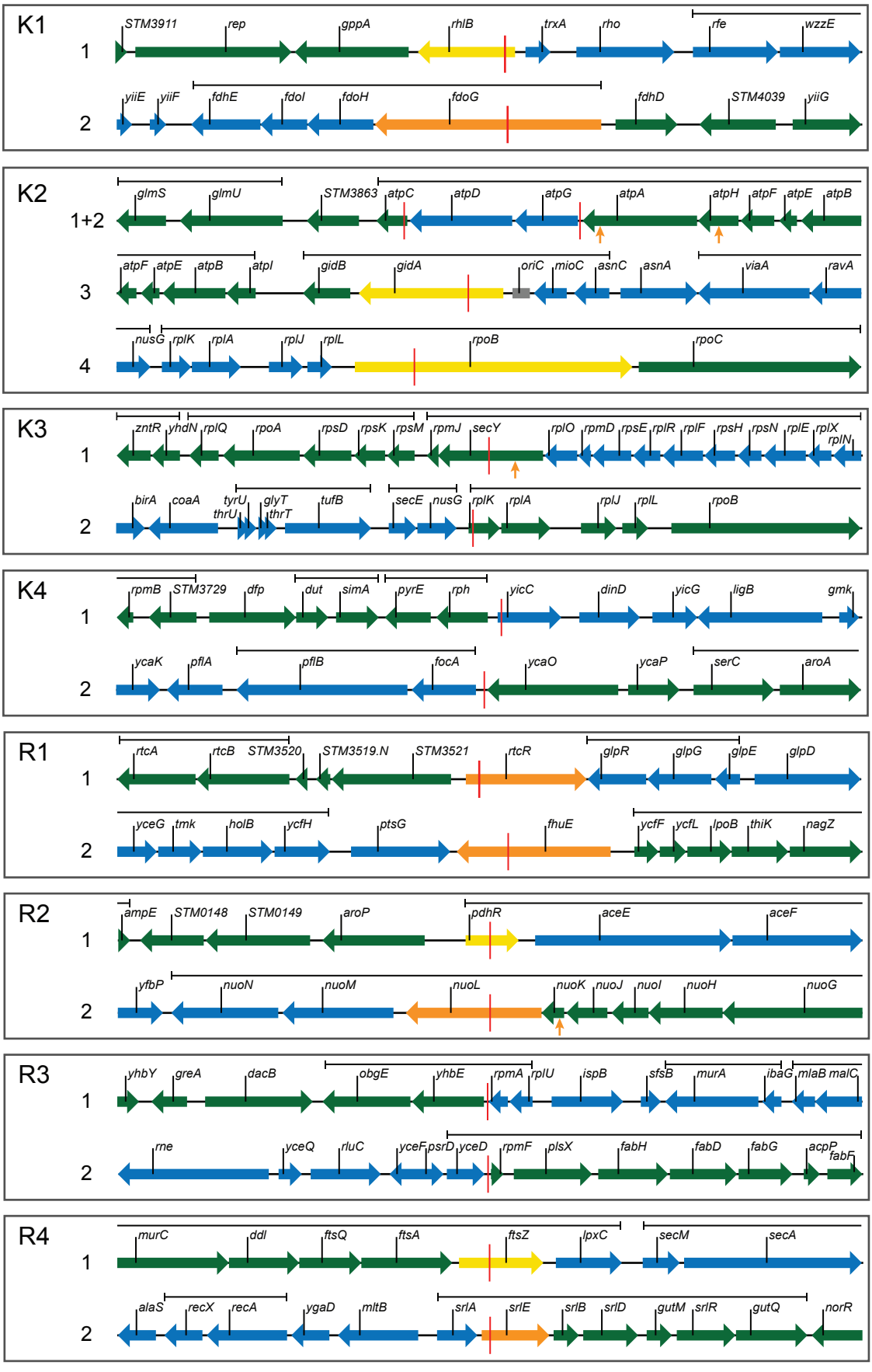
Supplementary FIG S2. Length of perfect sequence homology at the recombination junction.

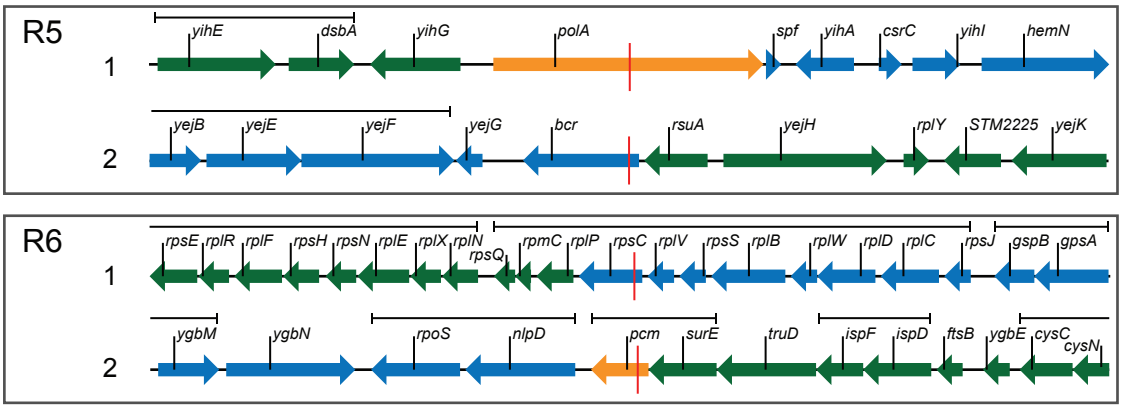


Supplementary FIG S3. Mechanism of hybrid chromosome formation. Donor DNA that has been conjugated into the recipient cell is processed by RecBCD (a). The processed DNA binds in a RecA-dependent manner to the homologous region on the recipient chromosome. The resulting formation of an imperfect heteroduplex DNA segment is strongly inhibited by the MusSLH mismatch repair system (b). Junction resolution leads to the formation of a hybrid chromosome that with an imperfect heteroduplex DNA segment (c). Mismatches within the heteroduplex can be repaired by the mismatch repair system (MMR) or maintained until the next round of replication resulting in a clear transition between donor and recipient DNA (d). Partial repair of the heteroduplex by the MMR (e) or repair of single mismatches by the base excision repair system (BER) (g) can lead to the formation of a transition that alternates between donor and recipient DNA (f, h).



Supplementary FIG S4. Nucleotide sequence alignment of the recombination junctions with alternating transitions. Green colour indicates *S.* Typhimurium and blue colour indicates *E. coli* sequence based on SNPs between the two species. The number above the alignment displays the distance to the closest recombination junction. The chromosomal locations of alternating transitions are shown in supplementary fig. S5.


K1
1
STM3911 rep gppA rhlB trxA rho rfe wzzE
2
yiiE yiiF fdhE fdoI fdoH fdoG fdhD STM4039 yiiG
K2
1+2
glmS glmU STM3863 atpC atpD atpG atpA atpH atpF atpE atpB
3
atpF atpE atpB atpI gidB gidA oriC mioC asnC asnA viaA ravA
4
nusG rplK rplA rplJ rplL rpoB rpoC
K3
1
zntR yhdN rplQ rpoA rpsD rpsK rpsM rpmJ secY rplO rpmD rpsE rplR rplF rpsH rpsN rplE rplX rplN
2
birA coaA tyrU thrU glyT thrT tufB secE nusG rplK rplA rplJ rplL rpoB
K4
1
rpmB STM3729 dfp dut simA pyrE rph yicC dinD yicG ligB gmk
2
ycaK pflA pflB focA ycaO ycaP serC aroA
R1
1
rtcA rtcB STM3520 STM3519.N STM3521 rtcR glpR glpG glpE glpD
2
yceG tmk holB ycfH ptsG fhuE ycfF ycfL lpoB thiK nagZ
R2
1
ampE STM0148 STM0149 aroP pdhR aceE aceF
2
yfbP nuoN nuoM nuoL nuoK nuoJ nuoI nuoH nuoG
R3
1
yhbY greA dacB obgE yhbE rpmA rplU ispB sfsB murA ibaG mlaB malC
2
rne yceQ rluC yceF psrD yceD rpmF plsX fabH fabD fabG acpP fabF
R4
1
murC ddl ftsQ ftsA ftsZ lpxC secM secA
2
alaS recX recA ygaD mltB srlA srlE srlB srlD gutM srlR gutQ norR



Supplementary FIG S5. Analysis of recombination junctions. Chromosome structure in a 10 kb window around each recombination junction. *S.* typhimurium genes are shown in green and *E. coli* genes are shown in blue. The red line indicates the first nucleotide that does not match the *S.* typhimurium sequence. Genes with a hybrid coding sequence are coloured based on their protein identity. Hybrid genes that encode proteins identical to the protein in either species are shown in green or blue, genes with a protein identity >99% to either species are shown in yellow, and genes with a protein identity <99% to either species are in orange. Orange arrows below genes indicate junctions with alternating transitions (see fig S4). Operon membership as indicated by the EcoCyc database (Keseler, et al. 2017) is indicated above the genes.

Supplementary Table S1. Frequency of selected colonies as a function of conjugation parameters.

Donor	Recipient	Time / h	Selection	Frequency
CH6459	TH6767	3	TET + SPT	$8.1\ 10^{-7}$
CH6459	TH6767	6	TET + SPT	$4.6\ 10^{-6}$
CH6459	TH6767	12	TET + SPT	$9.6\ 10^{-6}$
CH6941	TH10684	3	KAN + CHL	$2.8\ 10^{-7}$
CH6941	TH10684	6	KAN + CHL	$2.8\ 10^{-6}$
CH6941	TH10684	12	KAN + CHL	$9.5\ 10^{-6}$
CH6941	TH10684	3	RIF + CHL	$< 3.0\ 10^{-9}$
CH6941	TH10684	6	RIF + CHL	$1.8\ 10^{-8}$
CH6941	TH10684	12	RIF + CHL	$8.6\ 10^{-8}$

Supplementary Table S2. Strain list.

Strain	Species	Genotype & comments	Source
TH6767	*S.* Typhimurium	*metA22 metE551 trpD2 ilv-452 leu- pro-* (leaky) *hsdLT6 hsdSA29 hsdB strA120 eutR<>spt*	TT24693 John Roth, UC Davis
TH10684	*S.* Typhimurium	*metA22 metE551 trpD2 ilv-452 leu- pro-* (leaky) *hsdLT6 hsdSA29 hsdB strA120 eutR<>spt ydgI<>cat*	This study
CH6459	*E. coli*	HfrKL14(PO68) transfers CW F inserted at IS2 next to *yqiG relA1 thiE1 spoT1 ilvD691*::Tn*10*	BW6159 Barry Wanner, HMS
CH6941	*E. coli*	HfrKL14(PO68) transfers CW F inserted at IS2 next to *yqiG relA1 thiE1 spoT1 ilvD691*::Tn*10 yiiF<>kan ymdA<>rif queE<>gen*	This study
T1 (CH7196)	Hybrid	CH6459 *fis* nt138 - *ilvC* nt1401 replaces TH6767 *fis* nt138 - *ilvC* nt1401	This study
T2 (CH7194)	Hybrid	CH6459 *sspA* nt120 - *engB* nt-26 replaces TH6767 *sspA* nt120 - *engB* nt-26	This study
T3 (CH7195)	Hybrid	CH6459 *gltB* nt2481 – *glnA* nt1317 replaces TH6767 *gltB* nt2481 – *glnA* nt1317	This study
T4 (CH7193)	Hybrid	CH6459 *infB* nt2535 – *ulaE* nt705 replaces TH6767 *infB* nt2535 – *ulaE* nt705	This study
K1 (CH7200)	Hybrid	CH6941 *rhlB* nt150 - *fdoG* nt1443 replaces TH10684 *rhlB* nt150 - *fdoG* nt1443	This study
K2 (CH7198)	Hybrid	CH6941 *atpC* nt54 - *atpG* nt-21 replaces TH10684 *atpC* nt54 - *atpG* nt-21 AND CH6941 *gidA* nt384 - *rpoB* nt1326 replaces TH10684 *gidA* nt384 - *rpoB* nt1326	This study

Supplementary Table S2. Continued.

K3 (CH7201)	Hybrid	CH6941 *secY* nt747 - *rplK* nt54 replaces TH10684 *secY* nt747 - *rplK* nt54	This study
K4 (CH7206)	Hybrid	CH6941 *yicC* nt54 - *focA* nt-146 replaces TH10684 *yicC* nt54 - *focA* nt-146	This study
R1 (CH7210)	Hybrid	CH6941 *rtcR* nt279 - *fhuE* nt1437 replaces TH10684 *rtcR* nt279 – *fhuE* nt1422	This study
R2 (CH7207)	Hybrid	CH6941 *pdhR* nt363 - *nuoL* nt732 replaces TH10684 *pdhR* nt363 - *nuoL* nt732	This study
R3 (CH7211)	Hybrid	CH6941 *yhbE* nt-87 - *rpmF* nt-31 replaces TH10684 *yhbE* nt-91 - *rpmF* nt-31	This study
R4 (CH7203)	Hybrid	CH6941 *ftsZ* nt369 - *srlE* nt108 replaces TH10684 *ftsZ* nt369 - *srlE* nt108	This study
R5 (CH7208)	Hybrid	CH6941 *polA* nt1362 - *bcr* nt90 replaces TH10684 *polA* nt1362 - *bcr* nt90	This study
R6 (CH7202)	Hybrid	CH6941 *rpsC* nt153 - *pcm* nt159 replaces TH10684 *rpsC* nt153 - *pcm* nt159	This study
CH7231	Hybrid	Evolved T1-1 [a]	This study
CH7232	Hybrid	Evolved T1-2 [a]	This study
CH7233	Hybrid	Evolved T1-3 [a]	This study
CH7234	Hybrid	Evolved T1-4 [a]	This study
CH7236	Hybrid	Evolved T1-5 [a]	This study
CH7237	Hybrid	Evolved T1-6 [a]	This study
CH7218	Hybrid	Evolved T2-1 [a]	This study
CH7219	Hybrid	Evolved T2-2 [a]	This study
CH7220	Hybrid	Evolved T2-3 [a]	This study

Supplementary Table S2. Continued.

K3 (CH7201)	Hybrid	CH6941 *secY* nt747 - *rplK* nt54 replaces TH10684 *secY* nt747 - *rplK* nt54	This study
K4 (CH7206)	Hybrid	CH6941 *yicC* nt54 - *focA* nt-146 replaces TH10684 *yicC* nt54 - *focA* nt-146	This study
R1 (CH7210)	Hybrid	CH6941 *rtcR* nt279 - *fhuE* nt1437 replaces TH10684 *rtcR* nt279 – *fhuE* nt1422	This study
R2 (CH7207)	Hybrid	CH6941 *pdhR* nt363 - *nuoL* nt732 replaces TH10684 *pdhR* nt363 - *nuoL* nt732	This study
R3 (CH7211)	Hybrid	CH6941 *yhbE* nt-87 - *rpmF* nt-31 replaces TH10684 *yhbE* nt-91 - *rpmF* nt-31	This study
R4 (CH7203)	Hybrid	CH6941 *ftsZ* nt369 - *srlE* nt108 replaces TH10684 *ftsZ* nt369 - *srlE* nt108	This study
R5 (CH7208)	Hybrid	CH6941 *polA* nt1362 - *bcr* nt90 replaces TH10684 *polA* nt1362 - *bcr* nt90	This study
R6 (CH7202)	Hybrid	CH6941 *rpsC* nt153 - *pcm* nt159 replaces TH10684 *rpsC* nt153 - *pcm* nt159	This study
CH7231	Hybrid	Evolved T1-1 [a]	This study
CH7232	Hybrid	Evolved T1-2 [a]	This study
CH7233	Hybrid	Evolved T1-3 [a]	This study
CH7234	Hybrid	Evolved T1-4 [a]	This study
CH7236	Hybrid	Evolved T1-5 [a]	This study
CH7237	Hybrid	Evolved T1-6 [a]	This study
CH7218	Hybrid	Evolved T2-1 [a]	This study
CH7219	Hybrid	Evolved T2-2 [a]	This study
CH7220	Hybrid	Evolved T2-3 [a]	This study

Supplementary Table S2. Continued

CH7221	Hybrid	Evolved T2-4 [a]	This study
CH7223	Hybrid	Evolved T2-5 [a]	This study
CH7222	Hybrid	Evolved T2-6 [a]	This study
CH7224	Hybrid	Evolved T3-1 [a]	This study
CH7225	Hybrid	Evolved T3-2 [a]	This study
CH7227	Hybrid	Evolved T3-3 [a]	This study
CH7228	Hybrid	Evolved T3-4 [a]	This study
CH7226	Hybrid	Evolved T3-5 [a]	This study
CH7212	Hybrid	Evolved T4-1 [a]	This study
CH7213	Hybrid	Evolved T4-2 [a]	This study
CH7230	Hybrid	Evolved T4-3 [a]	This study
CH7214	Hybrid	Evolved T4-4 [a]	This study
CH7215	Hybrid	Evolved T4-5 [a]	This study
CH7216	Hybrid	Evolved T4-6 [a]	This study
CH7217	Hybrid	Evolved T4-7 [a]	This study
CH9713	Hybrid	T1, CH7196 Δ*relA*	This study
CH9716	Hybrid	T2, CH7194 Δ*relA*	This study
CH9714	Hybrid	T3, CH7195 Δ*relA*	This study
CH9715	Hybrid	T4, CH7193 Δ*relA*	This study

[a] Full list of mutations acquired during experimental evolution in supplementary table S5.

Supplementary Table S3. Relative growth rates of hybrid strains. [a]

Strain	Relative growth rate ± SD in LB	Relative growth rate ± SD in M9
T1 (CH7196)	0.66 ± 0.01	0.45 ± 0.09
T2 (CH7194)	0.75 ± 0.01	0.41 ± 0.03
T3 (CH7195)	0.75 ± 0.02	0.45 ± 0.01
T4 (CH7193)	0.83 ± 0.02	0.29 ± 0.02
K1 (CH7200)	0.95 ± 0.02	0.98 ± 0.08
K2 (CH7198)	0.82 ± 0.01	0.89 ± 0.04
K3 (CH7201)	0.73 ± 0.02	0.60 ± 0.03
K4 (CH7206)	0.85 ± 0.02	0.72 ± 0.05
R1 (CH7210)	0.82 ± 0.02	0.87 ± 0.01
R2 (CH7207)	1.14 ± 0.02	1.33 ± 0.09
R3 (CH7211)	0.84 ± 0.01	0.78 ± 0.03
R4 (CH7203)	1.17 ± 0.06	1.20 ± 0.10
R5 (CH7208)	1.12 ± 0.05	1.22 ± 0.05
R6 (CH7202)	1.09 ± 0.05	1.03 ± 0.14

[a] Exponential growth rate ± standard deviation relative to TH6767.

Supplementary Table S4. Relative growth rates of evolved hybrid strains. [a]

Strain	Relative growth rate ± SD [b]
T1 (CH7196)	0.66 ± 0.01
T1-1 (CH7231)	0.95 ± 0.02 ***
T1-2 (CH7232)	0.89 ± 0.01 ***
T1-3 (CH7233)	0.91 ± 0.02 ***
T1-4 (CH7234)	0.89 ± 0.05 ***
T1-5 (CH7236)	0.88 ± 0.01 ***
T1-6 (CH7237)	0.89 ± 0.02 ***
T2 (CH7194)	0.75 ± 0.01
T2-1 (CH7218)	0.96 ± 0.03 ***
T2-2 (CH7219)	0.80 ± 0.00 ***
T2-3 (CH7220)	1.01 ± 0.03 ***
T2-4 (CH7221)	0.91 ± 0.03 ***
T2-5 (CH7223)	0.91 ± 0.01 ***
T2-6 (CH7222)	0.93 ± 0.01 ***
T3 (CH7195)	0.75 ± 0.02
T3-1 (CH7224)	0.91 ± 0.02 ***
T3-2 (CH7225)	0.86 ± 0.02 ***
T3-3 (CH7227)	0.89 ± 0.02 ***
T3-4 (CH7228)	0.88 ± 0.01 ***
T3-5 (CH7226)	0.90 ± 0.02 ***
T4 (CH7193)	0.83 ± 0.02
T4-1 (CH7212)	0.85 ± 0.01 n.s.
T4-2 (CH7213)	0.91 ± 0.06 *
T4-3 (CH7230)	0.85 ± 0.04 n.s.
T4-4 (CH7214)	0.80 ± 0.01 *
T4-5 (CH7215)	0.86 ± 0.05 n.s.
T4-6 (CH7216)	0.95 ± 0.02 ***
T4-7 (CH7217)	0.91 ± 0.03 **

[a] Exponential growth rate ± standard deviation relative to TH6767.

[b] n.s.: non-significant, *: P<0.05, **: P<0.01, ***: P<0.001 (two-tailed t-test compared with unevolved parental strain)

Supplementary Table S5. Full list of acquired mutations in evolved hybrids.

T1-1 (CH7231)

relA nt211 ins IS10 [a]	*hycD* nt403 ins IS10	*STM0530* Ala195Val
STM2503 nt728 ins IS10 [b]	*mdlA* Gly461Ser	*STM0710* ntG468A
mutS nt2290 ins IS10 [d]	*menE* ntG927A	*STM3022* nt576 ins IS10
glpT Tyr406fs [e]	*mig-3* Gly134fs	*STM3350* Glu202fs
nudE Pro54Ser [e]	*mukB* Asp234Gly	*sucA* Thr307Ala
treB nt543 ins T [e]	*oppA* ntT-233C	*thrA* Cys280fs
aldB ntG-79A	*pgi* His303Tyr	*ugpA* nt130 ins IS10
argA ntT709C	*phnT* Val68Ala	*uup* Asn174Ser
bcfD Ser259Gly	*phoQ* Arg291His	*vacB* ntC1932T
carB Thr740Ala	*proV* nt449 ins IS10	*yacC* ntC-98T
cbiO Lys143fs	*queF* Asp262Gly	*ybbO* Leu117Pro
cfa nt292 ins IS10	*rhaB* Tyr163Cys	*yeaG* Gly115fs
cysQ ntT474C	*rpoB* Ser621Pro	*yebE* Asp204Gly
dmsA nt1017 ins IS10	*ssaJ* Ser62Pro	*yeeO* Ala17Thr
dnaA Arg398Cys	*stdB* Leu819Phe	*ygiF* Leu78fs
envE nt-244 ins G	*STM0042* ntC36T	*yliJ* Leu52Val
gst ΔntA-51	*STM0281* Thr328Ala	*yncC* nt161 ins IS10
*hpaX nt*C1128T	*STM0356* ntA-67G	

T1-2 (CH7232)

relA nt184 ins IS10 [a]	*treB* nt543 ins T [e]	*yceE* nt658 ins IS10
yegE nt1913 ins IS10 [b]	*cysA* nt158 ins IS10	*yceO* nt-4 ins IS10
STM0030 nt721 ins IS10 [e]	*sopE2* nt-43 ins IS10	*yfbG* Phe537Val
glpT nt854 ins IS10 [e]	*ybdR* nt339 ins IS10	yjjY nt22 ins IS10

T1-3 (CH7233)

relA Val325Met [a]	*msbA* ntA651G	*STM3083* nt1197 ins IS10
mutS nt1154 ins IS10 [d]	*nifJ* ntG3411A	*STM3127* Ala24fs
arcB Thr469Ile [e]	*oat* Glu38Lys	*STM3133* Ala270fs
glpT His147Arg [e]	*pheR* A-75G	*STM4213* Val9Ala
treB nt543 ins T [e]	*rnd* ntG798A	*STM4495* Val122Met
bcsA ntG2052A	STM0054 Arg258His	*yajR* Ala385Thr
bcsB ntG-4A	*STM0062* Asp122Gly	*ycfQ* downstream nt25 ΔT
cysJ Tyr270His	*STM0306* nt703 ins IS10	*ydcP* Asp94Gly
hisA Ile223Thr	*STM0716* Leu74fs	*yeeA* ntG-102A
kdsA ntG519A	*STM1472* T-143C	ynfC Val107fs
metG ntG1392A	*STM1786* nt-43 ins C	*ytfM* Ser199Leu
mpl Leu16fs	*STM2006* nt-166 ins G	

T1-4 (CH7234)

relA nt 211 ins IS10 [a]	*glpT* nt 207 ins IS10 [e]	*wecH* nt599 ins IS10
arcB nt 34 ins IS10 [e]	*rfbK* nt899 ins IS10	*yjeJ* nt-125 ins IS10
csgC nt114 ins IS10 [e]	*stcC* nt 1515 ins IS10	*yjeJ* nt837 ins IS10
fljA nt407 ins IS10 [e]	*tdcE* nt 344 ins IS10	

T1-5 (CH7236)

relA nt211 ins IS10 [a]	*treB* nt545 ins C [e]	*STM0893* nt350 ins IS10
arcB Ala69Val [e]	*STM0335* downstream nt3 ins IS10	*yehZ* nt308 ins IS10
glpT nt149 ins IS10 [e]		

Supplementary Table S5. Continued.

T1-6 (CH7237)

relA nt381 ins IS10 [a]	*hypF* Cys109Arg	*STM1586* Pro281Leu
mutL nt365 ins IS10 [d]	*ilvI* ntA-85G	*STM1849* ntG342A
arcB Thr267Ala [e]	*lpp* nt-166 ins G	*STM2400* nt239 ins IS10
glpT nt474 ins IS10 [e]	*osmY* nt-136 ins IS10	*STM2503* ntT-58C
treB nt543 ins T [e]	*pagO* Pro63Ser	*STM3031* ntA-556G
adiY nt433 ins IS10	*pduT* nt142 ins IS10	*STM3251* Asn87Ser
ampG Ser123Pro	*rbsK* ntA-71G	*STM3334* ntC969T
araB Gly281Ser	*rhaT* ntA306G	*syd* ntC375T
aspV downstream nt39 ins A	*rhlB* ntC564T	*ybdN* Ser135fs
cvpA ntC-230T	*rnc* ntT-50C	*yeeO* Thr367Ala
cysN Val235Ala	*rumA* Ala212Val	*yfbQ* nt-356 ins C
dmsA Asp450Asn	STM0277 nt32 ins IS10	*ygaA* Val386Ala
eutG Asn299Ser	*STM1188* nt434 ins IS10	*yhgA* Arg278His
gltJ ntC480T	*STM1260* Val28fs	*yjgB* ntG-58A
gspE Gly359Asp	STM1441 Val153Ala	

T2-1 (CH7218)

relA Gln246stop [a]	*hemG* ntA81G	*STM1493* ntC540T
STM0551 Met70Val [b]	*hisS* ntG168A	*STM1537* ntG543A
mutS nt1154 ins IS10 [d]	*mltB* nt-47 ins IS10	*STM1636* Leu31Pro
fimW ntA-111G [e]	*murC* Val401Ile	*STM1755* Asp29Glu
glpT Ser70Pro [e]	*phoB* nt-76ΔT	*STM3126* Asp46fs
ompF nt744 ins IS10 [e]	*rne* downstream nt55 ins A	*STM3166.S* Tyr138His
treB nt543 insT [e]	*rpoA* Asp305Gly	*STM4011* Arg84Pro
amtB ntA1191G	*sodC* nt505 ins IS10	*STM4199* ntC459T
artQ Gly69Ser	*solA* Ala220fs	*STM4540.S* nt342 ins IS10
asnT ntT65C	*stdB* Arg806Trp	*sucD* Asn187Ser
bioA ntG426A	*stiA* ntC270 T	*tatB* Asp118Asn
fepG Ala136Thr	*STM0294* Ala3Thr	*yaiW* Met362Val
gltB Asp296Asn	*STM0409* Pro94Ser	*yecE* ntG684A
gltJ Ala124Thr	*STM04870* nt158 ins IS10	*yejG* nt247 ins IS10
glxK Arg294stop	*STM1002* Gly30Glu	*yfeZ* Thr136Ile
guaA ntG480A	*STM1109* nt253 ins IS10	*yhjA* Als188Thr
hemA Thr361Ala	*STM1147* nt-3 ΔG	*yjbH* Ser15Gly

T2-2 (CH7219)

relA nt211 ins IS10 [a]	*fhlA* nt940 ins IS10	*STM2574* nt446 ins IS10
glpT nt358 ins IS10 [e]	*lasT* nt-370 ins IS10	*STM4218* nt-23 ins IS10
treB nt543 ins T [e]	*nfi* Ala167Glu	*STM4218* nt-31 ins IS10
dipZ nt1192 ins IS10	*stjB* nt580 ins IS10	

Supplementary Table S5. Continued.

T2-3 (CH7220)		
relA Tyr201Cys [a]	*hcp* Gly208Ser	*STM2186* Tyr153Cys
yeiE ntT819C [c]	*hemY* Gly335Asp	STM2240 Tyr247His
mutS nt1154 ins IS10 [d]	*ilvD* Asn205Ser	*STM2532* ntT4698C
mutM Arg109Cys [d]	*ilvG* Asp156Asn	*STM2788* ntT981C
glpT Thr306Ala [e]	*nadC* Arg32His	*STM4261* Ala1066Val
nudE Pro142Ser [e]	*nlpB* Met241Val	*thrA* ntA1401G
astB Ala27Val	*phnT* Arg168His	*ttrB* Cys114fs
baeS Glu456stop	*ppdB* Asp177Gly	*vacB* Arg580Cys
crp Ala85Thr	*prpR* ntA-99T	*wcaC* Ser46fs
dacD Lys4Arg	*rffH* ntC60T	*ycbK* Ser131fs
dsbB Gly61Ser	*STM0098* nt414 ins IS10	*ydgJ* Trp47stop
dsbB Met102Thr	*STM0162* Thr134Ala	*ydiL* C-137T
envE nt-244 ins G	*STM0307* Val26Ala	yecA Pro60fs
envZ ntT291C	STM1515 downstream nt260 ΔT	ygeD Arg214Cys
eutC Thr270fs	*STM1558* Asp135Gly	*ygiE* downstream ntC31T
feoB ntT300C	*STM1843* ntC1263T	*yliJ* ntC153T
fxsA Ile79Val	*STM1864* downstream ntT91C	*ypfG* Ala120Val
grxA nt-81 ins IS10	*STM2006* nt-166 ins G	
T2-4 (CH7221)		
relA nt211 ins IS10 [a]	*hycD* Ala218Val	*sicA* Phe110fs
mutH nt466 ins IS10 [d]	*hycG* ntT219C	*STM0257* Leu171Pro
arcB Thr397Met [e]	*hypB* Ala147Thr	STM0520 Leu170fs
glpT Ser150Leu [e]	*mppA* nt946 ins IS10	*STM0895* nt68 ins IS10
treB nt543 ins T [e]	*nrfA* Gln346Arg	*STM1147* nt-3 ΔG
yjeP Arg278Gln	*oafA* nt628 ins IS10	*STM1253* nt-77 ins IS10
aroP ntG1227A	*otsA* Asp15fs	*STM2192* Ile136Thr
cadA Glu361Lys	*pbpC* Ile62fs	*STM2453* downstream nt46 ΔGC
cpsG Gln148stop	*ratB* ntG5682A	*STM4076* nt560 ins IS10
cysA Phe133Ser	*rfaZ* Met227fs	*STM4495* Arg1081His
fieF Ile245Thr	shdA Asp766Asn	*ydeI* downstream nt260 ΔT
folB Ala76Val	*hycD* Ala218Val	
T2-5 (CH7223)		
relA nt211 ins IS10 [a]	*nudE* nt431 ins IS10 [e]	*STM0081* downstream nt3 ins IS10
lrhA nt-53 ins IS10 [c]	*treB* nt543 insT [e]	*STM1669* nt1871 ins IS10
glpT nt10 ins IS10 [e]	*fdnH* nt141 ins IS10	

Supplementary Table S5. Continued.

T2-6 (CH7222)		
lrhA nt-53 ins IS10 [c]	*invC* Thr166Ile	*STM1097* nt130 ins IS10
mutS nt143 ins IS10 [d]	*manA* Ala322Thr	*STM2585* ntΔ-504--436 ins IS10
glpT Tyr393Cys [e]	*ompS* nt143 ins IS10	*wcaH* Ala61fs
treB nt543 ins T [e]	*pheP* nt285 ins IS10	*ybiP* ntC1272T
ahpC ntA486G	*pmrF* Pro111Leu	*ydiN* nt381 ins IS10
allC Ala253Thr	*proA* Val117Ala	*yebA* nt279 ins IS10
citG Ala98Pro	*rcsC* ntC2076T	*yebG* ntT72C
cstA ntA2015G	*rpoA* Asp305Gly	*yedE* Gly64Ser
fadB Met145Thr	*serU* ntT-2C	*yejF* ntG801A
fumC Leu433Val	*sprB* ntC-237T	*yejG* nt247 ins IS10
gcvR Asp104Asn	*stcC* Phe16Leu	*yhbE* ntG914A
glnA nt-114 ins C	*STM0201* downstream nt1 ins G	*yhbT* nt-11 ins IS10
glpA Gly332Asp	*STM04610* nt-22 ins G	*yibH* nt726 ins IS10
hdeD downstream nt196 ins IS10	*STM0557* nt858 ins IS10	*yjdL* Ser121Asn
hisH Tyr159Cys	*STM0560* ntG-29A	*yjiO* ntC-77T
hpaB ntG1366A		
T3-1 (CH7224)		
relA nt211 ins IS10 [a]	*gcvP* ntG2352A	*srfC* Ser143Pro
STM0014 nt557 ins IS10 [c]	*gltF* Ala159Thr	*stdB* Ala630Val
mutS nt1154 ins IS10 [d]	*guaB* Arg206Cys	*STM0266* Gln35fs
arcB Ala69Val [e]	*hisH* Arg86His	*STM0347* nt117 ins IS10
fliY Asn106Lys [e]	*idnO* Tyr190His	*STM0383* Ala55fs
glpT Ala235fs [e]	*katE* Pro514Leu	*STM0725* Ile260Val
treB nt543 ins T [e]	*otsA* Asp15fs	*STM0761* ntT525C
alaV ntG-47A	*parE* Asp614Val	STM1082 Thr115Ala
bamA ntA2307G	*pduL* ntG333A	*STM1656* downstream ntT35C
cadA Gln455Arg	*phnT* Gly56Glu	*STM2689* ntG10846A
ccmF Ala91fs	*pmbA* ntC873T	*tatD* ntC243T
celF nt148 ins IS10	*rbsR* ntC870T	tktB downstream ntG4A
crp Ile113Thr	*rnpB* nt271 ins C	*tus* ntC669T
csrB ntT129C	*rspB* downstream nt133 ins IS10	*uspB* nt-125 ΔT
dsbA Ser147Gly	*sbcD* Asp205Asn	*yoaA* nt550 ins IS10
flgL Ala55Val	*smf* Arg363Gly	
T3-2 (CH7225)		
relA nt804 ins IS10 [a]	*sopA* nt2120 ins IS10	*STM3154* nt370 ins IS10
glpT nt1198 ins IS10 [e]	*stjC* nt136 ins IS10	*STM4102* nt740 ins IS10
gspD nt36 ins IS10	*STM0035* nt53 ins IS10	*ybiS* nt431 ins IS10
lasT nt-365 ins IS10	*STM0277* nt143 ins IS10	*yfdH* nt593 ins IS10
rfbF nt46 ins IS10	*STM3026* nt186 ins IS10	*yjbE* nt-415 ins IS10
rfbG nt 857 ins IS10	*STM3052* nt-376 ins IS10	

Supplementary Table S5. Continued.

T3-3 (CH7227)

relA nt211 ins IS10 [a]	*fucR* ntG-10A	*STM1539* Ser159fs
yfeA ntC1752T [b]	*fumC* Ala24fs	*STM1557* Pro45Ser
STM0764 nt165 ins IS10 [c]	*gspB* Val89Ala	*STM1657* ntA516G
mutS nt1154 ins IS10 [d]	*hisP* Leu168fs	*STM2239* Ala14fs
mutM ntA-18G [d]	*kbl* Ser285Pro	*STM2529* Asn89fs
arcB Ala69Val [e]	*mltC* Thr291Ala	*STM2816* Thr249Ala
glpT Ser159Leu [e]	*parC* Thr249Ala	*STM3031* ntT-560C
nudE Gln130stop [e]	*pflB* Val476Ala	*STM3220* Ala340Val
treB nt543 ins T [e]	*ratA* Thr239Ala	*STM4208* Glu104Lys
apbA Trp165stop	*rbbA* ntT1026C	sucC ntA936G
argH Val267Ala	*rcsA* nt52 ins IS10	*tktB* Gly539Ser
asrC Arg134His	*rfaE* Thr29Ala	*yafS* Arg187fs
aroE nt-54 ins IS10	*rfbF* Gly130Ser	ybdR His11Arg
cspB nt-183 ins IS10	*STM0035* ntC180T	*ycaM* nt1123 ins IS10
cobS Val47fs	*STM0054* Glu397Lys	*yeeF* downstream ntT27C
corC Asp284Gly	*STM0182* ntC483T	*yejK* Als53Val
cysG Asp367Gly	*STM0717* Pro58Ser	*yidJ* nt1183 ins IS10
eutH Ser44Pro	*STM0721* ntC102T	*yjgD* ntC243T
foxA Tyr481fs	*STM0867* Trp203Arg	*yjgN* nt532 ins IS10

T3-4 (CH7228)

relA nt211 ins IS10 [a]	*ilvD* Ser579fs	*STM1849* nt478 ins IS10
adrA Met51Val [b]	*iscA* Thr75Ala	*STM2007* Trp432stop
mutS nt143 ins IS10 [d]	*moaD* Asn56Ser	*STM2691* Leu328fs
glpT nt45 ins IS10 [e]	*pdxH* ntT520C	*STM3133* Ala270fs
treB nt543 ins T [e]	*pyrD* Leu194Ser	*trg* nt423 ΔT
caiT Pro17fs	*recR* ntG225A	*ycfU* ntA-135G
cfa nt921 ins IS10	*rfbU* Pro70Ser	*yejG* Pro81Leu
cypD Ala436Thr	*rpoB* Glu876Lys	*ygjU* ntT-166C
eutB ntG618A	*shdA* ntG5730A	*yicL* Gly268Arg
fabZ ntT-4C	*stfC* Glu740Gly	*yihF* nt292 ins IS10
fadJ Gly116fs	*sthB* Gln57fs	*yrdE* nt82 ins IS10
glnK Ala111Val	*STM0353* Glu426fs	*ytfN* Ala362Thr
gltB Val69Ala	*STM0433* Gly157Glu	*ytfN* Thr343Ala
ilvD ntA-20G	*STM1147* nt-3 ΔG	

T3-5 (CH7226)

relA nt211 ins IS10 [a]	*pagD* nt-313 ins IS10	*STM2208* nt467 ins IS10
glpT nt-114 ins IS10 [e]	*res* nt1106 ins IS10	*STM2235* nt96 ins IS10
nudE nt343 ins IS10 [e]	*STM0011* nt330 ins IS10	*STM2621* nt190 ins IS10
treB nt543 ins T [e]	*STM0652* nt184 ins IS10	*uvrY* nt58 ins IS10
adhP nt62 ins IS10	*STM1228* nt699 ins IS10	*yejG* nt199 ins IS10
celF nt913 ins IS10	*STM1255* nt36 ins IS10	

T4-1 (CH7212)

relA nt211 ins IS10 [a]	*oafA* nt1455 ins IS10	*STM0288* nt482 ins IS10
yegE nt1912 ins IS10 [b]	*ompL* nt683 ins IS10	*STM1667* nt337 ins IS10
glpT nt609 ins IS10 [e]	*rfbC* nt132 ins IS10	*virK* nt463 ins IS10
nudE nt343 ins IS10 [e]	*ssaC* nt1124 ins IS10	*ybjM* nt-70 ins IS10
treB nt532 ins C [e]	*ssaQ* nt85 ins IS10	*yeeZ* nt-43 ins IS10
gip nt479 ins IS10	*STM0082* downstream nt113 ins IS10	

Supplementary Table S5. Continued.

T4-2 (CH7213)		
relA nt211 ins IS10 [a]	*metI* Ala4Val	*STM2633.S* ntC327T
STM2503 nt-12 ins IS10 [b]	*mod* Asp155Val	*STM2678* Val20Ala
mutS nt143 ins IS10 [d]	*pgk* Arg183Cys	*STM2693* ntT-105C
arcB Arg278Trp [e]	*ppdC* ntT63C	*STM4539* Asp268Gly
glpT Ala380fs [e]	*pphB* Gly56Glu	*tctE* ntA744G
treB nt543 ins T [e]	*prpC* ntA561G	*xylF* Ala56Thr
alaS ntT1539C	*prpR* nt-104 ΔT	*yajI* Thr86Ala
cysG Phe49Leu	*pspG* Tyr17Ala	*ybhC* ntT591C
dnaE Glu313Gly	*purR* Arg264His	*ydeA* ΔLeu292
fdrA ntT1002C	*STM0029* ntG-101A	*yegO* Gly936Ser
fepE ntA840G	STM04630 ntA63G	*yhcO* downstream nt23 ΔC
gip nt479 ins IS10	*STM05680* nt197 ins IS10	*yidF* Tyr20Cys
hilA ntA1176G	*STM0719* Ala23Val	*yjdQ* ntC-135T
idnR nt423 ins IS10	*STM1147* nt-3 ΔG	*yjeF* ntC756T
ilvI ntA840G	*STM2344* ntC94T	
T4-3 (CH7230)		
relA nt211 ins IS10 [a]	*araE* nt41 ins IS10	*STM0149* nt791 ins IS10
lrhA nt-53 ins IS10 [c]	*frvR* nt1478 ins IS10	*STM0335* downstream nt3 ins IS10
STM0014 nt557 ins IS10 [c]	*pphA* nt112 ins IS10	*STM0699* nt69 ins IS10
STM0859 nt320 ins IS10 [c]	*prgH* nt494 - *prgK* nt179 ins IS10 (Δ1451nt)	*STM1133* nt173 ins IS10
fimC nt274 ins IS10 [e]	*rfbB* nt-114 ins IS10	*ydbH* nt2238 ins IS10
glpT nt122 ins IS10 [e]	*rfbF* nt95 ins IS10	*ydbL* nt169 ins IS10
nudE nt343 ins IS10 [e]	*sipA* nt224 ins IS10	*ydiF* nt280 ins IS10
treB nt543 ins T [e]	*srfJ* nt56 ins IS10	*yhcA* nt219 ins IS10
adiC nt40 ins IS10	*ssrA* nt2486 ins IS10	
T4-4 (CH7214)		
glpT nt413 ins IS10 [e]	*leuO* nt-27 ins IS10	*STM0868* nt793 ins IS10
cdd nt-7 ins IS10	*pagO* nt-957 ins IS10	*STM2132* nt127 ins IS10
glnX ntC35T	*rfc* nt-28 ins IS10	STM4463 nt-134 ins IS10
T4-5 (CH7215)		
relA nt211 ins IS10 [a]	*sseA* downstream nt2 ins IS10	*yegS* nt256 ins IS10
glpQ nt606 - *glpA* nt1062 Δ3303nt [e]	*STM0038* nt44 ins IS10	*yehS* nt-3 ins IS10
treB nt543 ins T [e]	*STM1865* downstream nt53 ins IS10	*yejG* nt247 ins IS10
sbcD nt334 ins IS10	*tdcA* nt555 ins IS10	*yfdZ* nt95 ins IS10
sopA nt262 ins IS10		

Supplementary Table S5. Continued.

T4-6 (CH7216)

relA nt879 ins IS10 [a]	*mglA* Lys315Glu	*STM2923 ntC-1T*
mutS nt143 ins IS10 [d]	*pduG* Leu186Pro	*STM3079*.S ntT-105C
glpT Met136fs [e]	*pflF* ntA1053G	*STM4417* ntC264T
nudE Phe31Leu [e]	*prgH* Val323fs	*STM4429* nt45 ins G
treB nt543 ins T [e]	*proX* Val225Ala	*thrU nt*C-165T
amtB ntA-25G	pyrB nt-22 ΔA	*waaA* Ala160Thr
aroE nt677 ins IS10	*ravA* nt-83 ins G	*yajR* Phe365Leu
ccmA Ala203fs	*rfbF* Pro211Leu	ybiP ntC930T
ddlA Cys138Arg	*rfc* nt-28 ins IS10	*yciG* nt-209 ins C
envF Gly187Ser	*rhlE* Ala409fs	*yciT* ntG363A
eutS Tyr80Cys	*scsB* Ser244Leu	*ydiF* Arg148Cys
exbD Thr137Ala	*shdA* Als52Val	*yegN* Trp719Arg
ftsN ntC873T	*sseC* nt998 ins IS10	*yejG* nt247 ins IS10
gpmM ntG237A	*sseJ* nt663 ins IS10	*yfgB* Val123Ile
gpmM Val415Ile	*stdA* Val179Ile	*ygdP* ntG348A
gshA ntG492A	*STM1019* nt247 ins IS10	*ygiF* Leu78fs
gutQ ntG891A	*STM1539* Arg279His	*yhaO* nt589 ins IS10
hemF Met70Ile	*STM1791* nt562 ins IS10	*yjbD* ntC-243T
manC Val166Ile	*STM1849* Ala813Thr	*yqeF* Ser31Gly
metL Glu720Gly	*STM2636* Asp24Gly	

T4-7 (CH7217)

relA Tyr228Cys [a]	melA Asn394Ser	*STM2689* ntT4232C
yfeA nt695 ins IS10 [b]	*pyrB* nt-22 ΔA	*STM2908* ntG132A
mutL Gly43Ser [d]	*rfbM* Leu445fs	*STM4435* Thr86fs
mutS nt1154 ins IS10 [d]	*rpsA* Pro335Ser	*STM4518* nt-16 ins G
arcB Met260Ile [e]	*ssaD* Ser234Asn	*thrA* Pro787Ser
glpT Arg27Cys [e]	*ssaP* Glu26Gly	*ybeU* Asp118Gly
treB nt543 ins T [e]	*STM0306* nt615 ins IS10	*ydeI* downstream nt260 ΔT
adiA downstream nt2 ins IS10	*STM0319* Thr39Ala	*yedI* nt134 ins IS10
cbiT Asp37Gly	*STM0564* nt1189 ins IS10	*yfiP* ntG-34A
eda ntA453G	*STM0720* Phe88fs	*yghB* Gly203Arg
foxA Asp118fs	*STM0911* nt416 ins IS10	ygjT nt-25 ΔTT
gabT Gly49Ser	*STM1390* Asn162Ser	*yieL* Pro129Ser
gpmB nt-9 ins A	*STM1665* nt655 ins IS10	*ymdF* Asn52Asp
gsiA Thr265Ala	*STM2372* nt-41 ΔC	*yncC* nt663 ins IS10
leuB Gly143Glu	*STM2651* ntT-14C	

[a] Genes related to ppGpp production

[b] Genes related to c-di-GMP production

[c] LysR-type genes

[d] Genes related to mutator phenotypes

[e] Genes related to media adaptation

Supplementary Table S6. Number of mutations and IS10 insertions found in evolved isolates.

Type of Mutation	**Mutator genotype** [a]		**p-value**[b]
	-	+	
IS10	12 ± 6	3 ± 3	0.03
non-IS10	1 ± 1	39 ± 7	< 0.001

[a] Changes in genes related to mutator phenotypes (supplementary table S5)

[b] Two-tailed Mann-Whitney U-test

Supplementary Table S7. Mutation frequency of evolved isolates.

Mutator genotype [a]	Mutation frequency	p-value[b]
+	$5\ 10^{-8} \pm 1.4\ 10^{-8}$	0.026
-	$4\ 10^{-9} \pm 7.3\ 10^{-9}$	

[a] Changes in genes related to mutator phenotypes (supplementary table S5)

[b] Two-tailed T-test

Supplementary Table S8. *relA* and *spoT* alleles present in parental strains and hybrids.

Strain	*relA* allele	*spoT* allele
Recipient (TH6767)	*relA*^{WT}	*spoT*^{WT}
Donor (CH6459)	*relA1*	*spoT1*
T1 (CH7196)	*relA*^{WT}	*spoT1*
T2 (CH7194)	*relA*^{WT}	*spoT1*
T3 (CH7195)	*relA*^{WT}	*spoT1*
T4 (CH7193)	*relA*^{WT}	*spoT1*
K1 (CH7200)	*relA*^{WT}	*spoT*^{WT}
K2 (CH7198)	*relA*^{WT}	*spoT*^{WT}
K3 (CH7201)	*relA*^{WT}	*spoT1*
K4 (CH7206)	*relA*^{WT}	*spoT1*
R1 (CH7210)	*relA*^{WT}	*spoT1*
R2 (CH7207)	*relA*^{WT}	*spoT*^{WT}
R3 (CH7211)	*relA*^{WT}	*spoT1*
R4 (CH7203)	*relA*^{WT}	*spoT*^{WT}
R5 (CH7208)	*relA*^{WT}	*spoT*^{WT}
R6 (CH7202)	*relA*^{WT}	*spoT1*

Supplementary Table S9. Growth data of hybrid strains as a function of RelA activity.

Hybrid	Relative growth rate ± SD [a, b]		Relative culture density ± SD [b, c]	
	relA	Δ*relA*	*relA*	Δ*relA*
T1 (CH7196)	0.76 ± 0.03	0.74 ± 0.03 [n.s.]	0.79 ± 0.01	0.93 ± 0.02 [***]
T2 (CH7194)	0.85 ± 0.03	0.88 ± 0.02 [n.s.]	0.77 ± 0.00	0.91 ± 0.01 [***]
T3 (CH7195)	0.86 ± 0.02	0.84 ± 0.03 [n.s.]	0.78 ± 0.02	0.90 ± 0.01 [***]
T4 (CH7193)	0.89 ± 0.04	0.94 ± 0.05 [n.s.]	0.89 ± 0.02	1.01 ± 0.03 [***]

[a] Exponential growth rate ± standard deviation relative to TH6767.

[b] [n.s.]: non-significant, [***]: P<0.001 (two-sided t-test compared with isogenic strain containing *relA*)

[c] OD_{600nm} after 18h of growth ± standard deviation relative to TH6767.

Supplementary Table S10. Oligonucleotides used in the study.

Name	Sequence 5' → 3'
insert_cat_fw	CCTGTTCCGGTCCTAATTACGTTATGCACACCAATGATGGCA CCAAACACCCCCCAAAAC
insert_cat_rv	TTATCGGTCTGGGGTTTACCGTCGGTCACAATGCTACGTTCA CACAACCACACCACACCA
insert_kan_fw	GGTGCAGCGTAATCTTCCTCGTGCGGACCTATTAAGGGAAC ACCAAACACCCCCCAAAAC
insert_kan_rv	TTGCTGTTTGCGATTGATTTATCAGGTATTGATCTACCGCCA CACAACCACACCACACCA
insert_rif_fw	GGCATGTGACGTCAGCACCCAGTCATCACCCGTAGAAATGC ACCAAACACCCCCCAAAAC
insert_rif_rv	AGTAGGTTTTGCCCGGAATAGAACCATTCTGTGGGCAGTTCA CACAACCACACCACACCA
test_cat_fw	CGCCTGTTCCGGTCCTAATT
test_cat_rv	GGGCTTTTGGCTTACGGTTG
test_kan_fw	CTGGAAAACGCCTCGCAAAA
test_kan_rv	TCCCATTACCATTCCTCGCG
test_rif_fw	AGGGGGCGTGATCCATTTTT
test_rif_rv	TTTCTGCTCAGCAACGTGAG
ΔrelA_fw	CTGGTTCAGCTTACCGAGCACCCGGCCCAGCACCTGCAGGC ACCAGCTTTATTAATATGTGTGTAGGCTGGAGCTGCTTC
ΔrelA_rv	ATGGTCGCGGTAAGAAGTGCACATATTAATAAAGCTGGTGC CTGCAGGTGCTGGGCCGGGGTGTAGGCTGGAGCTGCTTC