

Supporting Information

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Chemical Evolution of a Bacterium's Genome**

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Supporting Information

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Materials and Methods

Growth media and chemicals. Bacteria were grown in rich LB medium or in mineral standard medium (MS) supplemented with D-glucose (2 g/l) as a carbon source as previously described.^[1] Growth media were solidified with 15 g/l agar for the preparation of Petri dishes. Liquid and solid cultures were usually incubated at 37°C. Thymidine was added to the culture medium of *thyA*-deleted bacteria at 0.1 mM concentration. Conditional pulse feed experiments in the GM3 device were started with MS glucose medium containing 3 µM thymine as the relaxing medium and MS glucose medium containing 3 µM 5-chlorouracil as the stressing medium and continued after an initial 23 day cultivation phase with MS glucose medium containing 3 µM thymine plus 3 µM chlorouracil as relaxing medium, and MS glucose medium containing 3 µM chlorouracil as stressing medium. Temperature in the growth chambers was kept constant at 37°C throughout cultivation and the culture volume was set at 16 ml. When required, antibiotics were added at the following concentrations if not otherwise stated: ampicillin, 100 mg/l; erythromycin, 180 mg/l; chloramphenicol 25 mg/l; kanamycin, 30 mg/l. Chemicals were purchased from Sigma-Aldrich.

Bacterial strains and plasmids. The strains used and constructed in this study are all derivatives of the wild type *Escherichia coli* K12 strain MG1655. Their relevant genotypes and filiations are summarized in Table S1. The deletion of the *thyA* gene ($\Delta thyA::erm$) originated from strain $\beta 1308$.^[2] Growth of strains harbouring this deletion requires either thymidine or thymine in the culture medium. The deletion of the entire *deoCABD* operon which derives from strain BS253, was previously described.^[3] The *udp* and *trmA* coding sequences were excised from the chromosome of strain MG1655 and substituted by an excisable kanamycin or spectinomycin resistance cassette following a published procedure.^[4] Strain THY0 ($\Delta thyA::erm \Delta deo \Delta udp::kan$) was obtained by serial P1 transductions carried out according to the method of Miller.^[5] The obligate requirement for thymidine of THY0 bacteria was verified on MS glucose 0.2 % plates with or without thymidine at 0.1 mM. Thymine could not fulfill thymidylate requirement. The

Lactobacillus leichmannii ntd gene was PCR-amplified with Pfx Taq polymerase (Invitrogen) on chromosomal *L. leichmannii* DNA as template using suitable PCR primers to enable ligation into plasmid pSU18.^[6] PCR amplification, ligation, transformation and sequencing of the amplified gene were performed following standard procedures. The resulting plasmid, pIC15 (cat⁺ ntd⁺) was transformed into THY0 bacteria. Strain THY1 was selected on LB chloramphenicol plates containing 0.1 mM thymidine. Functional expression of the *ntd* gene was checked by growing THY1 bacteria on MS glucose plates supplemented with 10 μ M thymine.

THY1 bacteria were used to conduct two parallel conditional pulse feed experiments as described above. The generation time was fixed at 2 hours (culture I, 140 pulses of 950 μ l/day) and 4 hours (culture II, 140 pulses of 460 μ l/day), respectively. Strain CLU2 was derived from an isolated single colony obtained by plating 10 μ l of culture I on solid MS glc 5-chlorouracil medium after 164 days of cultivation, strain CLU4 was derived from culture II following the same procedure after 166 days of cultivation. CLU2 and CLU4 bacteria were subsequently grown on MS glc thymine plates, and colonies isolated to obtain THY2 and THY4 strains, respectively. Strain THY5 was obtained by P1 transduction of the Δ *trmA::aad* allele in THY4 bacteria grown in the presence of thymine. THY5 bacteria were subsequently grown on MS glc 5-chlorouracil plates and colonies isolated to obtain CLU5 bacteria.

Extraction and digestion of genomic DNA. Extraction of the genomic DNA of bacteria selected for growth in the presence of 5-chlorouracil was performed using the Genomic Tips kit (QIAGEN). The concentration of DNA was determined by measuring the absorbance at 260 nm (SAFAS Monaco spectrophotometer). The procedure for enzymatic digestion of DNA was adapted from.^[7,8] 20 μ g of purified DNA in 90 μ l 10 mM Tris (pH 8) were denatured in boiling water for 5 minutes in a microcentrifuge tube then chilled on ice. 10.5 μ l of 0.5 M sodium acetate buffer (pH 5.3) containing 20 mM ZnCl₂, and 5 μ l P1 Nuclease (Sigma, 1 mg/ml, 0.25 U/ μ l) were added. The samples were incubated for 2 hours at 37°C. Then 10 μ l of 0.1 M glycine hydrochloride buffer (pH 10.4) and 5 μ l bovine

intestinal mucosa alkaline phosphatase (Sigma, 1 mg/ml, 3.5 U/μl in glycine hydrochloride buffer) were added and the samples were incubated for 2 hours at 37°C. The samples were subsequently deaminated to obtain a complete conversion of deoxyadenosine into deoxyinosine thus avoiding non-reproducible results due to partial deamination of deoxyadenosine by deaminase impurities usually encountered with commercial phosphatase preparations.^[9] 5 μl adenosine deaminase (Sigma, 0.1 U/μl) were added to the nucleic acid digests and the samples were incubated for 1 hour at 30°C. The digests were then filtered by centrifugation in Nanosep 3K devices (Pall) in order to eliminate protein before injection into the HPLC column and stored at -20°C.

HPLC and LC/MS analysis. Samples (10 μl) of digested genomic DNA were injected into a Waters 2795 HPLC apparatus. Separations were performed on an Uptisphere 5ODB column (Interchim) at 30°C in a mobile phase consisting of 10% methanol - 90% buffer (12.5 mM citrate, 25 mM sodium acetate, 30 mM sodium hydroxide, pH 5.3) at a flow rate of 0.8 ml/min. The eluted products were detected in an UV detector (220-400 nm; Waters 996 Photodiode Array detector). The elution profile of each deoxynucleoside (deoxycytidine, deoxyinosine, deoxyguanosine, thymidine and deoxyadenosine) (1 mg/ml) was separately established using commercial standards under the same conditions of separation and UV detection.

LC/MS analyses were carried out using a LTQ/Orbitrap high resolution mass spectrometer coupled to an Accela LC system (Thermo-Fisher Scientific, Germany). Nucleosides were detected in the positive mode by a mass range of 50-1000 Th at a resolving power of 30 000 at $m/z = 400$. These compounds were detected using a Heated ESI source (150°C) at 4 kV with sheath, auxiliary and sweep gases set at 50, 45 and 5 arbitrary units, respectively.

Chromatographic separations of compounds were performed employing an Aquity UPLC BEH C18 column (100 x 2.1 mm, 1.7 μm; Waters) column at a flow rate of 0.4 ml/min. The column was eluted with 10 mM ammonium acetate adjusted to pH 4 with 0.1% formic acid (A) and methanol (B). The column was held at 100% A for 2 min and subsequently

ramped to 100% B over 6 min, followed by 2 min at 100% B. The column and samples were maintained at temperatures of 50°C and 4°C, respectively.

Preparation and sequencing of genomic DNA libraries. Mate-paired libraries for 454-Titanium sequencing (454 Life Sciences) were generated from 15 µg of genomic DNA by employing the HydroShear technology (Genomic Solutions). The resulting 8 kbp DNA fragments were end-repaired, column-purified, ligated and circularized as described by the furnisher. The circularized constructs containing the genomic DNA were column-purified and sheared using Adaptive Focused Acoustic Technology (Covaris, Inc.). Sheared constructs were end-repaired and ligated with 454 paired end sequencing adapters and single stranded 454 libraries generated and sequenced on a Genome FLX Sequencer using a 454-Titanium sequencing kit. Approximately 300 000 mate-pair reads were produced for each genomic DNA sequenced, with an average length of 356 bp for strain CLU2 and 378 bp for strain CLU4, yielding a coverage of about 24x for the two strains.

Single-end libraries for sequencing with the Illumina Genome Analyzer IIX (Illumina Inc.) were generated following a protocol recommended by the supplier. Genomic DNA (1 µg) was sheared using Adaptive Focused Acoustic Technology (Covaris, Inc.) to generate fragments of 350 bp average length. Fragments were end-repaired, column-purified, tailed with Taq polymerase, column-purified and ligated with Illumina forked paired end sequencing adapters (Illumina Inc.) and again column-purified. Fragments were size-selected (350-500 bp, agarose gel) and column-purified. Three ng of each library were PCR amplified and sequenced. For each strain sequenced, about 27 million valid single-end reads (L=72 bp) were generated, yielding a coverage of 420x with respect to the reference genome (*E. coli* K12 wt strain MG1655).

Mutation Analysis. A script based on ssaha2 (<http://www.sanger.ac.uk/resources/software/ssaha2/>) alignment software has been implemented to allow the detection of Single Nucleotide Variations (SNVs, i.e. SNPs and microindels) between a batch of short

reads and a reference sequence. SNVs discovery is achieved in three main steps: (i) Preparation of data, i.e. files conversion, duplicate reads removal and possibly paired-end reads split. (ii) Mapping of reads onto a reference molecule using the ssaha2 package.^[10] This package combines the SSAHA searching algorithm (Sequence Search and Alignment by Hashing Algorithm and the cross_match software (<http://www.phrap.org>)). Regions of high similarity are identified by SSAHA and aligned using a banded Smith-Waterman-Gotoh algorithm.^[11,12] (iii) Assessment of significance of detected SNVs based on the coverage and the quality of bases of reads displaying this deviation from the reference sequence.

Genome analysis

A number of genes involved in DNA replication, repair mechanisms and DNA precursor metabolism were found to bear missense or nonsense mutations in THY2 (Table S3) but stayed unchanged in THY4. One example of such a locus mutated only in THY2 is the gene *mutL* coding for one of the components of the methyl-directed mismatch repair system of *E. coli*.^[13,14] The glycine codon 142 of *mutL* is changed to glutamate in the THY2-version of the gene. *E. coli* strains deficient in MutL activity show an increased mutation frequency. Expression of the *mutL* gene from THY2 in an *E. coli* strain harbouring a *mutL* chromosomal deletion did not suppress the mutator phenotype caused by the deletion, while the expression of the wild type *mutL* gene in this strain lowered the mutation frequency to wild type level (data not shown). Thus, the appearance of the G142E *mutL* missense mutation and probably mutations in some other genes controlling genetic variability in *E. coli* might have favoured the adaptation process of CLU2, as often observed with long-term evolution of *E. coli* B to common metabolic regimes.^[15] Whether and how the harsher CLU2 selection conditions (2h generation time for CLU2, 4h for CLU4) promoted the selection of the MutL mutator phenotype and a massive accumulation of point mutations remains to be investigated.

In both strains the chromosomal region between the *hrpA* and the *ydeP* gene loci was found to be deleted (102 kbp deletion in THY4, 150 kbp deletion in THY2 spanning from *uspF* to

ydeP, Table 1). In addition to this shared deletion three other deletions spanning 6.6, 27 and 38 kb, respectively, have been fixed during evolution of CLU4 as revealed by sequencing the THY4 genome. It is not known whether gene elimination in the deletion spans resulted from positive selection in adapting to increasing chlorouracil usage. Chlorouracil substitution might have caused the neutral fixation of numerous deletions through misadjustments of the nucleoid, replication, recombination or repair machineries. Alternatively, the sheer sparing of thymine through genome shrinking caused fixation of these deletions.^[16] These important issues should be addressed in future studies.

A 846 kb inversion covering almost 20 percent of the genome is found in the chromosome of THY4 between the *hns/tdk* intergenic region and the *asmA* gene. The inversion disrupts the *asmA* gene (coding for the predicted assembly protein AsmA) and brings the *tdk* gene for thymidine kinase downstream of the *dcd* and *udk* genes, encoding respectively dCTP deaminase and uridine kinase. Thymidine kinase plays a crucial role in adaptation to chlorouracil since it phosphorylates both thymidine and the competing substrate, chlorodeoxyuridine, both nucleosides resulting from deoxyribosylation commanded by the *ntd* gene (see Figure S1). It can thus be surmised that this large inversion, whose result was to integrate the expression of the *tdk* gene in the *udk-dcd* operon, was selected during adaptation of the CLU4 lineage to chlorouracil usage, a hypothesis that awaits to be tested experimentally.

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Table S1: Genotypes of *E. coli* strains.

Strain	Genotype	Strain construction / selection
MG1655	F- l- rph-1	
G2297	$\Delta trmA::aad$	MG1655 derivative (laboratory collection)
THY0	$\Delta thyA::erm \Delta deo \Delta udp::kan$	MG1655 derivative (laboratory collection), thymine auxotroph
THY1	$\Delta thyA::erm \Delta deo \Delta udp::kan$ pIC15 <i>cat+</i> <i>ntd+</i> <i>Lactobacillus leichmannii</i>	Transformation of THY0 with plasmid pIC15, thymine auxotroph
CLU2	$\Delta thyA::erm \Delta deo \Delta udp::kan$ pIC15 <i>cat+</i> <i>ntd+</i> <i>Lactobacillus leichmannii</i>	THY1 derivative selected in continuous culture, chlorouracil auxotroph (2000 generations, generation time = 2 hours)
THY2	$\Delta thyA::erm \Delta deo \Delta udp::kan$ pIC15 <i>cat+</i> <i>ntd+</i> <i>Lactobacillus leichmannii</i>	CLU2 grown in MS glucose T medium
CLU4	$\Delta thyA::erm \Delta deo \Delta udp::kan$ pIC15 <i>cat+</i> <i>ntd+</i> <i>Lactobacillus leichmannii</i>	THY1 derivative selected in continuous culture, chlorouracil auxotroph (1000 generations, generation time = 4 hours)
THY4	$\Delta thyA::erm \Delta deo \Delta udp::kan$ pIC15 <i>cat+</i> <i>ntd+</i> <i>Lactobacillus leichmannii</i>	CLU4 grown in MS glucose T medium
THY5	$\Delta thyA::erm \Delta deo \Delta udp::kan$ $\Delta trmA::aad$ pIC15 <i>cat+</i> <i>ntd+</i> <i>Lactobacillus leichmannii</i>	Transduction of THY4 with a P1 lysate of G2297
CLU5	$\Delta thyA::erm \Delta deo \Delta udp::kan$ $\Delta trmA::aad$ pIC15 <i>cat+</i> <i>ntd+</i> <i>Lactobacillus leichmannii</i>	THY5 grown in MS glucose chlorouracil medium

Strain nomenclature: The three-letter prefix of each strain name (THY or CLU) indicates the genome's chemical state made from either an exogenous thymine supply (THY) or an

exogenous chlorouracil supply (CLU). The suffix digit (e.g., 2) corresponds to a given genotype whether it is in the thymine or chlorouracil chemical state (e.g., THY2 and CLU2).

Table S2: Concentration of thymidine and 5-chloro-2'-deoxyuridine ($d\chi$) in DNA digests of *E. coli* (expressed in μM , experiments were done twice). Concentrations were calculated from the Mass Spectrometry peak areas using calibrating curves (Figure S3, S4). The fraction of the dT concentration over the sum of the $d\chi + dT$ concentration is also shown.

Sample	[dT] (μM)	[$d\chi$] (μM)	[dT] / ([$d\chi$] + [dT])
CLU2	16.07	166.02	0.088
CLU4	14.29	117.32	0.108
CLU5	2.00	123.57	0.016
THY1	316.12	0	1

Table S3: Intragenic single nucleotide variations in strain THY2. Gene affected, chromosomal position, position in the gene and the nature of the mutation are listed together with its consequence on the encoded amino acid. * indicates a stop codon; blanks in the "Amino acid change" column indicate either frameshift mutations or mutations in non translated RNAs.

Gene	Chromosomal position	Gene position	Mutation	Amino acid change
thrA	2328	1992	T/C	G664G
yaaJ	6893	1067	A/G	V356A
dnaK	12240	78	G/A	V26V
nhaA	17931	443	T/C	V148A
ribF	22131	725	A/G	N242S
ileS	23706	1316	A/G	K439R
ileS	24190	1800	C/T	G600G
fkpB	26024	199	C/T	P67S
carA	30373	723	T/C	G241G
carB	31779	963	A/G	K321K
caiC	36575	1265	A/G	V422A
caiB	38655	461	T/C	N154S
caiB	39066	50	A/G	V17A
caiA	39720	667	A/G	F223L
caiT	41891	41	A/G	V14A
fixC	44740	561	A/G	E187E
fixC	45280	1101	G/A	P367P
kefC	48292	524	C/T	S175L
ksgA	52089	342	C/T	L114L
pdxA	52647	770	T/C	N257S
polB	65768	13	C/T	G5S
araC	70509	123	A/G	G41G
araC	70819	433	G/A	G145R
thiQ	72295	633	C/T	Q211Q
thiQ	72393	535	G/A	Q179*
tbpA	75195	286	A/G	F96L
setA	78776	1156	A/G	T386A
leuA	83170	360	T/C	E120E
ilvI	86616	987	A/G	E329E
ilvI	86694	1065	A/G	E355E
ilvH	87397	41	G/A	G14D
fruR	88176	149	A/G	N50S
mraZ	89705	72	A/G	E24E
ftsI	91849	437	A/G	D146G
murD	97553	467	T/C	V156A
ftsW	98486	84	C/T	G28G

Table S3: Intragenic single nucleotide variations in strain THY2.

Gene	Chromosomal position	Gene position	Mutation	Amino acid change
ftsQ	103649	495	C/T	G165G
ftsQ	103871	717	A/G	K239K
secM	108177	473	A/G	Q158R
secA	109233	955	C/T	L319L
yacF	112059	541	A/G	F181L
coaE	112614	606	T/C	S202S
guaC	114016	573	A/G	K191K
guaC	114239	796	T/C	F266L
hofB	116029	1071	A/G	H357H
hofB	116119	981	A/G	R327R
aceE	125045	2029	C/T	R677C
yacH	130488	773	T/C	N258S
yacL	134496	109	G/A	A37T
speE	136310	155	A/G	V52A
yacC	136760	158	C/T	G53E
gcd	139363	1863	A/G	G621G
gcd	139700	1526	A/G	V509A
yadE	145458	378	A/G	V126V
yadC	150737	217	A/G	Y73H
yadN	156800	84	A/G	G28G
pcnB	158863	264	C/T	T88T
yadB	159503	610	G/A	L204L
dksA	160526	79	G/A	P27S
hrpB	162749	645	A/G	L215L
hrpB	163244	1140	A/G	Q380Q
mrcB	165811	1082	A/G	N361S
mrcB	166256	1527	T/C	G509G
hemL	173781	1102	A/G	Y368H
hemL	174323	560	A/G	V187A
yadR	176891	282	T/C	R94R
dgt	180631	1395	T/C	D465D
frr	193211	340	A/G	T114A
ispU	195645	743	C/T	P248L
yaeT	199901	1974	C/T	S658S
yaeT	200327	2400	A/G	E800E
dnaE	205230	105	T/C	G35G
dnaE	205970	845	C/T	P282L
dnaE	206315	1190	T/C	V397A
dnaE	207147	2022	A/G	K674K
accA	209369	749	C/T	A250V
tilS	212439	109	A/G	T37A
tilS	213122	792	G/A	G264G
gloB	234545	238	T/C	T80A
yafM	247867	231	G/A	W77*
fhiA	249699	372	A/G	P124P
yafP	252826	118	G/A	A40T
yafP	252971	263	C/T	T88I

Table S3: Intragenic single nucleotide variations in strain THY2.

Gene	Chromosomal position	Gene position	Mutation	Amino acid change
prfH	253862	161	T/C	F54S
frsA	257260	734	C/T	S245F
crl	258147	319	C/T	L107L
phoE	258628	697	T/C	T233A
ykfG	263486	471	C/T	L157L
yafZ	266690	540	C/T	Q180Q
afuB	278070	331	T/C	T111A
yagF	284195	1771	A/G	T591A
yagH	286505	493	C/T	Q165*
yagK	291953	220	G/A	L74L
yagP	296817	199	A/G	L67L
yagT	301391	407	C/T	G136D
ykgJ	303314	93	A/G	G31G
ykgD	320140	690	T/C	R230R
ykgG	323451	470	G/A	S157N
betA	325293	1179	G/A	S393S
betI	328344	215	A/G	V72A
betT	329355	669	A/G	V223V
betT	330029	1343	G/A	G448D
yahF	337430	1429	A/G	T477A
yahK	343104	997	C/T	R333C
prpC	350233	998	A/G	N333S
prpD	350767	329	G/A	W110*
prpD	350770	332	G/A	G111D
prpE	352318	389	GC/--	
codB	354653	508	T/C	W170R
lacY	361759	645	T/C	E215E
lacZ	365437	93	A/G	H31H
mhpA	368061	227	T/C	V76A
yaiP	382367	793	G/A	L265F
hemB	388478	474	A/G	G158G
proC	404557	312	C/T	Q104Q
yaiA	406269	67	C/T	Q23*
sbcC	411869	3109	C/T	G1037S
malZ	423511	1773	G/A	G591G
tgt	425464	104	A/G	Y35C
tsx	430619	619	C/T	G207S
ribD	433197	519	G/A	S173S
ribE	434190	320	A/G	D107G
dxs	437616	1786	T/C	T596A
ispA	440240	86	T/C	N29S
yajL	442547	319	C/T	A107T
cyoA	450828	7	G/A	L3F
clpX	456726	77	G/A	R26H
ppiD	461528	390	C/T	D130D
ppiD	461689	551	C/T	A184V
ppiD	462439	1301	C/T	A434V

Table S3: Intragenic single nucleotide variations in strain THY2.

Gene	Chromosomal position	Gene position	Mutation	Amino acid change
mdlB	470092	233	C/T	A78V
tesB	473723	663	T/C	E221E
ylaB	476315	1527	T/C	L509L
acrA	484817	27	A/G	P9P
dnaX	491381	66	A/G	E22E
recR	494092	464	A/G	N155S
ybaL	501523	940	A/G	F314L
ybaP	506799	506	A/G	L169P
copA	509572	1032	A/G	G344G
ybaS	510971	107	T/C	V36A
ybaS	511299	435	A/G	Q145Q
cueR	513618	402	A/G	A134A
ybbN	516795	709	C/T	E237K
ybbN	517417	87	A/G	Y29Y
hyi	535480	547	C/T	Q183*
ybbW	537484	628	C/T	P210S
ybbW	537633	777	G/A	E259E
ybbY	540078	290	A/G	N97S
ybcF	550363	702	G/A	P234P
purK	551292	526	A/G	F176L
lpxH	552857	307	G/A	L103F
sfmA	557557	123	T/C	C41C
sfmD	559238	319	G/A	V107I
ylcG	573083	131	C/T	A44V
ybcS	577327	492	A/G	Q164Q
ybcY	581915	45	T/C	E15E
nfrA	588090	2088	C/T	M696I
nfrB	591547	855	A/G	R285R
cusR	594390	277	A/G	L93L
cusA	597985	49	C/T	L17L
pheP	601250	69	A/G	L23L
pheP	602323	1142	A/G	N381S
entF	616991	3612	T/C	S1204S
fepG	619987	425	A/G	I142T
fepB	622864	870	C/T	K290K
estA	629360	244	C/T	P82S
estA	630214	1098	T/C	G366G
ybdO	636515	327	A/G	S109S
ahpF	639606	631	G/A	D211N
ybdR	642177	867	A/-	
citX	646976	283	A/G	L95L
citF	647317	1478	A/G	V493A
citF	648243	552	G/A	C184C
citE	649190	524	C/T	R175H
citA	651855	398	G/A	S133N
dcuC	654335	857	A/G	F286S
dcuC	654791	401	A/G	V134A

Table S3: Intragenic single nucleotide variations in strain THY2.

Gene	Chromosomal position	Gene position	Mutation	Amino acid change
dcuC	654852	340	T/C	N114D
ybeM	657484	7	A/G	T3A
ybeF	659752	850	A/G	S284P
ybeF	660556	46	A/G	S16P
dacA	663050	137	G/A	A46V
mrdA	665825	1616	T/C	Y539C
ybeB	667984	276	T/C	E92E
leuS	673212	795	A/G	H265H
hscC	681538	1079	C/T	C360Y
ybeY	691406	159	A/G	S53S
ubiF	694429	106	C/T	H36Y
ubiF	694620	297	A/G	E99E
asnB	698115	286	T/C	T96A
nagD	698979	571	C/T	A191T
ybfC	735418	546	A/G	E182E
ybfL	736264	142	A/G	T48A
ybfL	737080	958	A/G	T320A
ybgA	738235	12	A/G	Q4Q
phr	739506	777	A/G	E259E
abrB	746901	92	A/G	L31P
ybgD	751893	126	A/G	C42C
gltA	752591	1101	A/G	G367G
sucA	758231	303	C/T	F101F
sucD	763485	83	C/T	A28V
mngA	766181	975	-/GG	
mngB	769548	2348	C/T	A783V
cydB	773325	1061	A/G	Y354C
tolR	775261	190	G/A	V64M
pal	778669	380	A/G	N127S
valZ	780333	43	G/A	
lysQ	780852	53	G/A	
gpmA	786182	637	G/A	P213S
galK	788847	356	C/T	G119D
modF	791675	1337	A/G	V446A
modE	793851	17	A/G	L6P
modA	794643	332	T/C	V111A
pgl	798420	612	A/G	E204E
ybhD	799229	633	A/G	G211G
ybhD	799820	42	C/T	S14S
bioA	807231	1250	A/G	V417A
bioF	809743	140	A/G	N47S
uvrB	814491	1743	A/G	E581E
uvrB	814571	1823	C/T	A608V
ybhN	820997	725	A/G	V242A
ybhP	823623	98	A/G	L33P
ybhF	826672	1533	G/A	V511V
ybiO	842793	1911	T/C	E637E

Table S3: Intragenic single nucleotide variations in strain THY2.

Gene	Chromosomal position	Gene position	Mutation	Amino acid change
glnQ	845209	478	A/G	F160L
glnQ	845533	154	C/T	E52K
ybiP	850405	1416	G/A	D472D
mntR	852566	161	-/G	
ybiT	855373	188	A/G	N63S
ybiW	861038	792	C/T	A264A
iaaA	866266	476	A/G	E159G
yliA	868096	1321	C/T	L441L
yliD	871505	393	C/T	G131G
yliF	875346	789	A/G	E263E
yliG	877181	78	A/G	R26R
cmr	883947	1052	C/T	A351V
ybjI	885315	40	A/G	F14L
ybjJ	886475	88	A/G	S30P
ybjL	887642	1401	G/A	G467G
ybjL	888080	963	T/C	Q321Q
ybjM	889380	69	T/C	F23F
ybjN	892394	215	C/T	A72V
potF	893146	140	C/T	A47V
potF	893824	818	C/T	A273V
ybjO	897374	163	T/C	F55L
artQ	900861	613	A/G	Y205H
ybjR	904442	307	A/G	S103G
ybjS	905102	875	A/G	V292A
poxB	908854	1419	A/G	G473G
hcr	910442	932	C/T	C311Y
hcp	912174	864	A/G	H288H
ybjD	916839	1144	T/C	L382L
ybjX	917893	451	G/A	Q151*
ybjX	917964	380	G/A	A127V
cydD	928914	1272	A/G	H424H
trxB	930734	540	A/G	G180G
trxB	931048	226	A/G	F76L
trxB	931176	98	A/G	V33A
ycaN	948150	642	G/A	V214V
ycaO	954949	907	A/G	F303L
serC	957063	188	T/C	V63A
serC	957949	1074	A/G	E358E
rpsA	962530	1313	A/G	E438G
rpsA	962556	1339	C/T	L447L
ycaI	963975	433	G/A	G145S
ycaR	970037	142	A/G	T48A
ycbJ	971168	194	C/T	A65V
mukF	974389	848	A/G	K283R
ssuB	992564	704	G/A	A235V
ssuC	993804	252	T/C	G84G
ssuA	995509	659	T/-	

Table S3: Intragenic single nucleotide variations in strain THY2.

Gene	Chromosomal position	Gene position	Mutation	Amino acid change
ycbT	1001448	419	-/G	
pyrD	1004349	359	T/C	V120A
ycbX	1006406	418	A/G	S140P
ycbX	1006650	174	A/G	T58T
ycbY	1007099	33	G/A	G11G
pqiB	1013608	1127	A/G	N376S
ycbZ	1016613	910	C/T	D304N
ompA	1018556	721	G/A	L241L
yccS	1022626	481	A/G	F161L
helD	1025367	1674	A/G	K558K
mgsA	1025918	321	A/G	R107R
hyaB	1032970	494	A/G	D165G
hyaB	1033467	991	T/C	F331L
appC	1037841	879	G/A	G293G
appA	1039903	64	G/A	A22T
yccC	1042819	615	A/G	R205R
yccZ	1044387	640	C/T	V214M
ymcE	1051231	162	A/G	V54V
yccM	1051882	704	A/G	V235A
torT	1056033	550	A/G	M184V
torC	1057439	133	A/G	T45A
torD	1061353	332	C/T	A111V
cbpA	1062458	541	T/C	T181A
yccE	1063977	719	A/G	N240S
agp	1065249	442	A/G	T148A
agp	1065321	514	C/T	L172F
agp	1065607	800	A/G	N267S
yccJ	1066117	198	G/A	G66G
rutA	1072407	828	T/C	E276E
putA	1077426	680	C/T	S227N
putP	1079339	812	G/A	R271H
pgaC	1086145	925	T/C	T309A
ycdT	1092620	522	C/T	S174S
csgG	1100228	680	A/G	V227A
ymdB	1105148	106	G/A	G36R
mdoC	1107296	869	A/G	V290A
mdoG	1108801	244	T/C	F82L
mdoG	1109288	731	A/G	K244R
mdtG	1114151	563	A/G	V188A
yceA	1116334	305	A/G	D102G
yceO	1118568	103	A/G	L35L
bssS	1120005	174	T/C	R58R
mviN	1128070	1009	T/C	F337L
flgA	1129953	134	A/G	V45A
flgE	1131989	193	A/G	T65A
flgF	1133260	236	A/G	D79G
flgF	1133564	540	T/C	R180R

Table S3: Intragenic single nucleotide variations in strain THY2.

Gene	Chromosomal position	Gene position	Mutation	Amino acid change
flgG	1134693	742	T/C	S248P
flgH	1135040	254	A/G	N85S
flgH	1135421	635	A/G	Y212C
flgJ	1137151	558	A/G	Q186Q
flgK	1138812	1212	G/A	T404T
rne	1141109	2482	A/G	L828L
rne	1142263	1328	A/G	V443A
rne	1142790	801	G/A	S267S
pabC	1152960	438	G/A	E146E
yceG	1154241	907	T/C	Y303H
ptsG	1158160	1069	T/C	F357L
fhuE	1158828	1947	G/A	G649G
ndh	1166571	1264	A/G	S422G
mfd	1170104	3084	T/C	K1028K
mfd	1170530	2658	T/C	E886E
lolE	1177046	504	T/C	I168I
potB	1182954	714	A/G	R238R
potA	1184665	153	A/G	P51P
ymfC	1194045	130	A/G	F44L
lit	1198434	517	A/G	T173A
lit	1198753	836	C/T	S279F
intE	1199866	164	G/A	A55V
ymfG	1200104	152	G/A	A51V
ymfP	1205790	308	A/G	D103G
ymfP	1206015	533	A/G	K178R
ycfK	1207012	289	C/G	L97V
ycgX	1212020	311	A/G	V104A
ycgG	1216845	295	A/G	T99A
ycgG	1217524	974	A/G	N325S
ymgH	1222884	197	A/G	N66S
ycgK	1226683	13	T/C	S5G
ycgL	1226999	96	A/-	
umuC	1230621	213	C/T	S71S
umuC	1230918	510	A/G	E170E
nhaB	1233270	671	T/C	N224S
nhaB	1233438	503	A/G	V168A
ycgB	1236205	260	T/C	H87R
dadA	1237952	1159	G/A	A387T
ldcA	1241962	342	A/G	G114G
ymgE	1244044	94	A/G	T32A
dhaR	1250308	20	A/G	N7S
dhaR	1251675	1387	A/G	T463A
ycgV	1254664	512	G/A	A171V
ychM	1258687	1340	C/T	R447H
hemA	1263726	790	C/T	R264C
prfA	1264686	452	C/T	A151V
prfA	1264873	639	A/G	E213E

Table S3: Intragenic single nucleotide variations in strain THY2.

Gene	Chromosomal position	Gene position	Mutation	Amino acid change
kdsA	1267707	320	A/G	D107G
chaA	1270968	105	T/C	T35T
narG	1279207	121	C/T	Q41*
narG	1279575	489	G/A	W163*
narG	1279694	608	G/A	G203D
narG	1280314	1228	A/G	T410A
narH	1283715	889	C/T	L297L
narH	1283797	971	C/T	A324V
rssA	1288819	391	A/G	T131A
rssA	1288890	462	A/G	G154G
hns	1291812	334	G/A	Q112*
hns	1291979	167	C/T	R56H
adhE	1296156	1189	T/C	N397D
oppA	1300670	1465	A/G	T489A
oppB	1300955	33	A/G	E11E
oppF	1304004	217	T/C	L73L
tonB	1309397	285	A/G	K95K
yciV	1322092	849	A/G	E283E
btuR	1326084	298	A/G	S100P
sohB	1328336	981	C/T	G327G
topA	1329346	275	T/C	V92A
topA	1331538	2467	G/A	E823K
yciS	1338396	130	T/C	L44L
yciM	1339643	1062	T/C	R354R
osmB	1341193	160	A/G	L54L
gmr	1343858	909	A/G	C303C
rnb	1346933	4	A/G	F2L
yciW	1347674	458	A/G	V153A
yciW	1347797	335	A/G	L112P
fabI	1348292	772	C/T	E258K
fabI	1348870	194	A/G	V65A
sapF	1350420	239	A/G	F80S
sapA	1354307	828	G/A	S276S
puuP	1357040	172	A/G	S58P
puuA	1358185	748	C/T	A250T
puuD	1359193	50	A/G	N17S
puuC	1361026	260	T/C	L87P
ycjM	1369537	1298	A/G	Y433C
ycjO	1371963	718	T/C	L240L
ycjQ	1373996	1010	A/G	D337G
ycjT	1377763	1856	C/T	A619V
tyrR	1385591	848	A/G	K283R
ycjG	1387457	504	G/A	V168V
ycjY	1389349	541	T/C	S181G
ynaJ	1395522	134	T/C	L45P
uspE	1396443	204	A/G	R68R
abgT	1398561	1237	C/T	A413T

Table S3: Intragenic single nucleotide variations in strain THY2.

Gene	Chromosomal position	Gene position	Mutation	Amino acid change
abgT	1399027	771	A/G	S257S
abgR	1403528	764	G/A	G255E
dbpA	1408415	881	T/C	V294A
ydaO	1409169	804	A/G	G268G
recT	1412396	422	A/G	V141A
racR	1417792	474	A/G	T158T
ydeR	1586124	197	C/T	G66D
yneE	1606791	256	-/C	
uxaB	1608444	261	A/G	R87R
yneI	1612477	251	T/C	N84S
yneK	1614000	214	A/G	T72A
ydeH	1621055	820	A/G	Y274H
ydeH	1621234	641	A/G	V214A
ydfZ	1627384	146	T/C	V49A
ydfJ	1629556	754	T/C	T252A
ydfU	1641576	753	T/C	E251E
flxA	1644606	178	G/A	V60I
dicC	1645790	85	T/C	S29G
rspB	1651386	554	T/C	N185S
ynfE	1657303	1211	G/A	S404N
ynfF	1659253	674	T/C	V225A
ynfF	1660546	1967	A/G	E656G
ynfF	1660644	2065	A/G	T689A
ynfK	1664713	531	T/C	G177G
ynfK	1665114	130	T/C	S44G
pntA	1675208	720	T/C	E240E
pntA	1675641	287	G/A	A96V
ydgl	1677692	112	G/A	A38T
ydgl	1677815	235	T/C	F79L
ydgl	1678821	1241	T/C	V414A
rstA	1680409	236	T/C	V79A
tus	1682291	9	T/C	R3R
ydgA	1687973	98	T/C	L33P
uidC	1689729	1147	T/C	M383V
uidC	1689783	1093	T/C	T365A
uidA	1693123	973	A/G	S325P
uidA	1693524	572	G/A	T191M
add	1700339	83	G/A	G28D
rsxA	1704049	259	T/C	F87L
rsxA	1704255	465	C/T	R155R
ydhl	1719185	137	G/A	G46D
ydhl	1719567	280	G/A	A94T
ydhl	1721143	999	T/C	R333R
lhr	1729841	2731	G/A	G911R
lhr	1730951	3841	G/A	A1281T
purR	1736255	388	T/C	L130L
purR	1736777	910	A/G	M304V

Table S3: Intragenic single nucleotide variations in strain THY2.

Gene	Chromosomal position	Gene position	Mutation	Amino acid change
ydhB	1736906	917	G/A	P306L
ydhC	1738887	953	T/C	V318A
mdtK	1742514	1034	A/G	N345S
mdtK	1742733	1253	C/T	P418L
ydhT	1747300	284	G/A	A95V
pykF	1754204	483	T/C	P161P
pykF	1754655	934	T/C	S312P
sufA	1762175	236	A/G	V79A
ydiJ	1764000	2710	G/A	P904S
ydiJ	1765751	959	A/G	V320A
ydiK	1767758	661	A/G	T221A
ydiN	1770884	349	G/A	A117T
ydiN	1770971	436	T/C	S146P
ydiN	1771369	834	G/A	R278R
ydiQ	1778319	679	A/G	T227A
ydiR	1779062	638	T/C	V213A
ydiS	1780465	1047	A/G	K349K
ydiD	1781935	935	T/C	V312A
pps	1784479	658	A/G	F220L
ydiU	1788066	1203	T/C	Q401Q
btuD	1791016	567	G/A	S189S
pheS	1796394	573	G/A	G191G
rplT	1797661	113	G/A	A38V
yniA	1806469	650	A/G	D217G
ydjN	1808980	23	A/G	N8S
chbA	1817573	257	A/G	V86A
chbC	1818144	1095	T/C	Q365Q
astE	1824455	493	C/T	D165N
astC	1829033	974	C/T	R325H
astC	1829680	327	G/A	N109N
xthA	1830674	223	G/A	A75T
ynjD	1837209	439	G/A	A147T
ynjE	1837996	506	A/G	K169R
selD	1845300	733	C/T	G245S
sppA	1846943	83	A/G	N28S
sppA	1848196	1336	G/A	D446N
ydjE	1851640	364	T/C	T122A
msrB	1860123	331	G/A	Q111*
yeaD	1861923	50	T/C	V17A
yeaI	1869000	592	T/C	L198L
yeaM	1873237	364	T/C	T122A
yeaU	1880279	344	T/C	V115A
yeaU	1880654	719	T/C	V240A
yeaV	1881216	5	T/C	V2A
yeaV	1882451	1240	A/G	T414A
yeaW	1883123	435	T/C	R145R
yeaW	1883379	691	A/G	T231A

Table S3: Intragenic single nucleotide variations in strain THY2.

Gene	Chromosomal position	Gene position	Mutation	Amino acid change
yeaX	1884184	316	T/C	F106L
yeaY	1888050	507	G/A	G169G
yeaY	1888398	159	A/G	G53G
yoaA	1889683	1577	T/C	D526G
yoaC	1892373	277	A/-	
pabB	1893677	849	T/C	P283P
sdaA	1896070	1115	G/A	G372D
rrmA	1904641	444	A/G	S148S
proQ	1913251	308	A/G	L103P
yebU	1918535	289	T/C	F97L
yebU	1919067	821	T/C	L274P
yebW	1920222	78	A/G	E26E
yebK	1934909	234	T/C	T78T
yebK	1935205	530	A/G	D177G
pykA	1936580	908	A/G	D303G
lpxM	1937642	576	T/C	K192K
yebA	1939068	592	C/T	A198T
aspS	1947289	1258	T/C	T420A
yecD	1949326	471	T/C	S157S
torY	1955336	821	G/A	A274V
yecM	1957535	336	T/C	E112E
argS	1958322	237	T/C	G79G
flhA	1962282	793	C/T	G265R
cheY	1965181	281	T/C	N94S
tar	1969922	794	A/G	V265A
tar	1970156	560	T/C	Y187C
flhD	1976131	100	A/G	S34P
araG	1982117	977	C/T	S326N
pgsA	1990363	479	G/A	A160V
uvrC	1991031	1700	T/C	K567R
uvrC	1992481	250	A/G	Y84H
uvrC	1992650	81	A/G	G27G
uvrY	1992868	516	T/C	E172E
uvrY	1993170	214	A/G	S72P
uvrY	1993271	113	T/C	D38G
fliC	2000258	1373	T/C	D458G
fliC	2000423	1208	G/A	A403V
fliC	2001301	330	A/G	S110S
fliS	2003566	240	A/G	E80E
fliF	2011268	16	G/A	A6T
fliH	2014526	635	T/C	V212A
fliI	2015753	1176	A/G	K392K
fliK	2017073	664	C/T	P222S
fliM	2019103	993	A/G	E331E
fliN	2019259	148	A/G	T50A
fliP	2020337	445	T/C	L149L
fliR	2020937	21	A/G	E7E

Table S3: Intragenic single nucleotide variations in strain THY2.

Gene	Chromosomal position	Gene position	Mutation	Amino acid change
rcsA	2022089	98	T/C	I33T
yedQ	2025150	892	T/C	T298A
yedI	2026670	721	A/G	F241L
yedI	2027002	389	T/C	K130R
yedA	2027923	361	A/G	T121A
yedJ	2030730	374	T/C	D125G
yedJ	2030874	230	G/A	A77V
yedY	2037614	113	T/C	L38S
yodB	2040402	11	T/C	F4S
yeeJ	2045415	2481	A/G	E827E
cbl	2058673	266	T/C	N89S
erfK	2060857	491	C/T	S164N
insH	2065298	48	T/C	Q16Q
dacD	2079911	661	A/G	L221L
yeeF	2084267	820	G/A	P274S
hisH	2092609	51	G/A	K17K
wbbK	2101822	712	T/C	T238A
rmlC	2108078	85	T/C	S29G
rmlA	2108404	640	A/G	S214P
rmlA	2108688	356	A/G	L119P
wcaL	2114960	192	T/C	E64E
wcaJ	2118873	706	G/A	L236L
wcaI	2122647	1124	A/G	L375P
wcaI	2123586	185	T/C	E62G
wcaB	2130322	258	C/T	A86A
wzc	2132637	1040	T/C	K347R
asmA	2138643	994	C/T	A332T
asmA	2139438	199	G/A	R67*
yegL	2151048	105	T/C	R35R
mdtB	2155473	2187	T/C	R729R
mdtC	2158584	2175	T/C	S725S
mdtD	2160787	1300	T/C	Y434H
yegQ	2164320	629	A/G	D210G
yegQ	2164329	638	A/G	N213S
gatA	2172969	103	T/C	T35A
yegX	2181348	326	T/C	D109G
thiD	2182142	397	C/T	V133I
rncA	2184037	99	C/T	H33H
yehB	2187789	1144	T/G	N382H
yehB	2187792	1141	T/A	N381Y
mrp	2192109	82	T/C	T28A
molR	2197839	496	G/A	D166N
molR	2198041	698	G/A	G233D
yehI	2200665	2365	G/A	G789R
yehL	2203097	480	T/C	R160R
yehL	2203479	862	T/C	S288P
yehM	2204381	665	T/C	L222S

Table S3: Intragenic single nucleotide variations in strain THY2.

Gene	Chromosomal position	Gene position	Mutation	Amino acid change
yehT	2210321	664	T/C	T222A
yehU	2211229	1438	T/C	T480A
yehW	2213808	691	A/G	F231L
yehX	2215059	371	T/C	E124G
yehY	2216253	327	G/A	G109G
bglX	2217735	2277	T/C	K759K
bglX	2218069	1943	T/C	Y648C
dld	2220225	19	A/G	T7A
cdd	2229965	100	A/G	T34A
folE	2241650	25	C/T	A9T
cirA	2243919	873	T/C	E291E
yeiC	2256876	443	G/A	A148V
yejA	2270563	178	G/A	A60T
yejA	2271186	801	T/C	G267G
yejH	2280180	1527	T/C	R509R
yejM	2282412	15	T/C	R5R
yejM	2283472	1075	T/C	L359L
yejO	2284510	2413	T/C	T805A
ccmH	2290221	212	T/C	Q71R
ccmH	2290340	93	T/C	E31E
ccmC	2293995	348	T/C	K116K
napH	2297029	572	A/G	V191A
napA	2299942	834	G/A	N278N
eco	2302310	384	T/C	R128R
mgo	2304364	413	A/G	V138A
yojL	2308720	837	A/G	R279R
ompC	2310458	314	T/C	D105G
ompC	2310757	15	T/C	V5V
rcsD	2312747	1238	A/G	N413S
rcsD	2312758	1249	T/C	Y417H
rcsC	2315923	1928	G/A	A643V
rcsC	2316519	1332	T/C	A444A
rcsC	2317201	650	T/C	N217S
rcsC	2317616	235	T/C	T79A
atoD	2322125	657	C/T	S219S
atoE	2323722	945	G/A	G315G
yfaQ	2327299	521	T/C	D174G
yfaQ	2327815	5	T/C	N2S
yfaS	2331427	998	T/C	N333S
yfaA	2334164	503	C/T	S168N
yfaL	2340150	2042	G/A	S681L
yfaL	2340986	1206	T/C	Q402Q
nrdB	2345479	74	A/G	N25S
glpB	2352590	304	T/C	L102L
glpC	2354362	820	C/T	L274L
yfaW	2359294	155	T/C	Y52C
yfaY	2360676	980	A/G	V327A

Table S3: Intragenic single nucleotide variations in strain THY2.

Gene	Chromosomal position	Gene position	Mutation	Amino acid change
yfaZ	2361771	527	A/G	V176A
ais	2363373	270	C/T	L90L
ais	2363438	205	T/C	T69A
yfbH	2368894	855	C/T	G285G
menB	2374087	755	T/C	N252S
menD	2377189	93	G/A	G31G
menF	2378260	406	C/T	D136N
menF	2378597	69	G/A	P23P
yfbL	2384156	275	C/T	P92L
yfbP	2387467	333	T/C	C111C
nuoH	2394941	524	T/C	N175S
nuoG	2395568	2626	C/T	A876T
nuoG	2395963	2231	T/C	N744S
nuoG	2396052	2142	A/G	R714R
nuoC	2401551	329	T/C	D110G
yfcF	2418342	166	T/C	T56A
yfcI	2421354	208	T/C	T70A
yfcI	2421549	13	T/C	T5A
dedD	2429282	425	T/C	K142R
dedD	2429426	281	T/C	N94S
trmC	2439791	6	A/G	K2K
aroC	2445436	60	C/T	G20G
prmB	2445963	500	T/C	D167G
yfcU	2452276	748	A/G	L250L
yfcU	2452363	661	T/C	T221A
yfcX	2457124	58	T/C	T20A
yfdC	2464004	682	A/G	T228A
intS	2464660	94	T/C	S32P
yfdP	2471884	343	T/C	Y115H
dsdX	2475925	57	A/G	V19V
dsdA	2478239	1016	G/A	G339D
emrY	2478862	1337	A/G	V446A
evgS	2483554	1159	A/G	T387A
yfdE	2486072	1119	T/C	E373E
yfdE	2487014	177	T/C	G59G
ypdB	2498630	226	T/C	F76L
ypdD	2501516	992	G/A	A331V
ypdF	2504340	315	C/T	A105A
glk	2507038	411	C/T	T137T
mntH	2510139	590	A/G	V197A
valU	2518967	15	G/A	
xapB	2521589	419	G/A	T140M
xapB	2521948	60	G/A	S20S
ligA	2526270	1929	T/C	E643E
ligA	2527496	703	G/A	L235L
ligA	2527911	288	T/C	K96K
cysM	2536700	906	C/T	G302G

Table S3: Intragenic single nucleotide variations in strain THY2.

Gene	Chromosomal position	Gene position	Mutation	Amino acid change
cysA	2538647	190	T/C	T64A
cysU	2539956	579	A/G	L193L
cysP	2541547	4	C/T	A2T
murP	2544802	108	T/C	G36G
murP	2545701	1007	T/C	F336S
murP	2545973	1279	T/C	S427P
murP	2546016	1322	T/C	I441T
yfeX	2547684	884	T/C	K295R
yfeX	2547725	843	T/C	K281K
hemF	2551312	66	G/A	Q22Q
yfeG	2552450	755	T/C	N252S
yfeG	2552881	324	T/C	G108G
yfeG	2553096	109	T/C	T37A
eutB	2556553	149	A/G	V50A
yffP	2560673	128	T/C	L43P
maeB	2575890	510	G/A	S170S
talA	2576711	24	A/G	K8K
acrD	2588271	2655	T/C	C885C
dapE	2590744	1116	G/A	Q372Q
ypfI	2592551	1331	T/C	H444R
purC	2595077	564	T/C	V188V
hyfD	2603859	1027	T/C	F343L
hyfJ	2609805	327	G/A	W109*
purM	2619833	615	T/C	G205G
ppx	2623790	654	T/C	G218G
yfgI	2628647	300	T/C	S100S
xseA	2633561	1308	C/T	G436G
der	2634275	1104	T/C	V368V
yfgL	2635920	755	A/G	V252A
yfgL	2636277	398	G/A	A133V
hisS	2638190	408	T/C	Q136Q
hisS	2638292	306	T/C	E102E
ndk	2642752	135	T/C	E45E
ndk	2642801	86	G/A	A29V
pbpC	2644605	743	G/A	A248V
yfhM	2646230	4080	T/C	E1360E
yfhM	2647743	2567	T/C	N856S
yfhM	2649633	677	T/C	D226G
ryfA	2652117	241	T/C	
hscA	2655284	1674	T/C	E558E
iscS	2658718	836	T/C	N279S
yfhR	2663232	848	A/G	D283G
hcaT	2664946	923	T/C	H308R
hcaT	2665860	9	C/T	L3L
hcaR	2666451	468	A/G	Y156Y
hcaE	2667308	255	C/T	C85C
yphB	2671952	759	T/C	E253E

Table S3: Intragenic single nucleotide variations in strain THY2.

Gene	Chromosomal position	Gene position	Mutation	Amino acid change
yphD	2674813	35	T/C	K12R
yphG	2678542	2226	C/T	E742E
yphG	2680504	264	T/C	K88K
yphH	2681595	711	G/A	Q237Q
glnB	2685108	323	T/C	D108G
yfhK	2688386	735	C/T	W245*
purL	2691108	2458	A/G	S820P
yfhH	2696895	115	C/T	Q39*
yfhH	2697421	641	G/T	G214V
era	2701349	60	G/A	G20G
lepB	2702522	810	G/T	F270L
lepA	2703455	1692	C/T	L564L
nadB	2708748	307	T/C	L103L
srnB	2711055	138	T/C	G46G
c0362	2712321	136	T/C	
yfiQ	2718253	279	C/T	G93G
clpB	2730389	1807	A/G	F603L
clpB	2730501	1695	T/C	E565E
yfiH	2732565	492	C/-	
yfiO	2734586	419	C/T	A140V
pheA	2736608	842	T/C	V281A
yfiN	2741248	844	T/C	L282L
rplS	2742487	66	C/T	P22P
yfjB	2748964	112	A/G	I38V
yfjB	2749471	619	A/G	T207A
recN	2751365	1549	C/T	H517Y
intA	2755200	1020	T/C	R340R
yfjH	2756464	159	T/C	E53E
yfjL	2762408	768	T/C	K256K
yfjP	2766415	684	T/C	H228H
yfjP	2766428	697	A/G	T233A
yfjQ	2767381	695	G/A	G232E
ypjK	2768537	71	G/A	S24N
yfjU	2769953	224	A/G	I75T
ypjA	2777018	3731	G/A	A1244V
ypjA	2777361	3388	C/T	V1130I
ypjA	2777776	2973	G/A	S991S
ypjA	2778422	2327	A/G	L776P
ypjA	2779067	1682	T/C	N561S
gabD	2789839	545	C/T	A182V
gabP	2793646	1372	C/T	Q458*
ygaU	2794422	387	T/C	K129K
ygaU	2794544	265	T/C	T89A
ygaP	2795654	113	C/T	A38V
ygaM	2798416	261	T/C	R87R
nrdE	2800556	1187	G/A	S396N
proW	2804503	472	T/C	F158L

Table S3: Intragenic single nucleotide variations in strain THY2.

Gene	Chromosomal position	Gene position	Mutation	Amino acid change
proW	2804938	907	A/G	M303V
ygaY	2806928	295	T/C	L99L
emrA	2810402	954	T/C	R318R
mltB	2823159	440	A/G	V147A
hydN	2835611	517	G/A	L173L
hydN	2835727	401	C/T	C134Y
ascG	2836385	905	T/C	E302G
hypA	2848789	121	A/G	T41A
hypB	2849357	335	G/A	S112N
hypB	2849755	733	T/C	Y245H
mutS	2856914	1800	G/A	P600P
ygbI	2859185	102	T/C	E34E
ygbN	2863199	77	T/C	V26A
ygbN	2863371	249	T/C	G83G
truD	2868421	906	C/T	L302L
truD	2868544	783	G/A	G261G
ispD	2870506	7	T/C	T3A
cysD	2874346	6	A/T	D2E
iap	2875387	785	A/G	D262G
ygbT	2877092	703	T/C	I235V
ygcJ	2879547	618	T/C	L206L
ygcB	2883548	1694	T/C	N565S
ygcB	2883881	1361	A/G	V454A
ygcB	2884044	1198	A/G	W400R
ygcB	2884700	542	G/A	A181V
cysJ	2888826	1095	A/G	R365R
cysJ	2888897	1024	G/A	L342L
cysJ	2889021	900	T/C	E300E
cysJ	2889336	585	G/A	S195S
ygcW	2897547	749	T/C	H250R
eno	2905814	150	T/C	E50E
pyrG	2907208	481	C/T	A161T
mazG	2908121	587	T/C	D196G
barA	2914512	1434	T/C	R478R
gudP	2918844	1279	T/C	I427V
gudP	2920103	20	G/A	A7V
truC	2921486	321	T/C	E107E
truC	2921573	234	T/C	E78E
ygdH	2925416	1087	T/C	F363L
sdaB	2928601	1004	C/T	A335V
fucA	2931284	427	C/T	A143T
fucP	2932377	121	G/A	A41T
fucI	2934033	428	T/C	V143A
fucI	2934073	468	A/G	E156E
fucI	2934582	977	A/G	N326S
fucI	2935342	1737	C/T	Y579Y
fucR	2937847	458	T/C	V153A

Table S3: Intragenic single nucleotide variations in strain THY2.

Gene	Chromosomal position	Gene position	Mutation	Amino acid change
csdA	2941478	120	A/G	Q40Q
csdA	2942315	957	T/C	R319R
recD	2950266	218	C/T	S73N
recB	2953584	442	A/G	F148L
ptr	2954548	2359	A/G	F787L
ptr	2955071	1836	A/G	R612R
recC	2957829	2622	T/C	G874G
recC	2960000	451	T/C	T151A
ptsP	2964382	2075	T/C	N692S
ptsP	2966285	172	G/A	L58L
nudH	2966745	255	A/G	R85R
ygdQ	2968860	419	G/A	S140N
tas	2969645	27	T/C	S9S
tas	2969685	67	T/C	F23L
tas	2970549	931	A/G	T311A
omrB	2974334	74	-/A	
galR	2975384	764	T/C	V255A
kduI	2981560	587	T/C	H196R
kduI	2981606	541	G/A	L181L
kduI	2982007	140	A/G	V47A
ygeI	2991672	13	A/G	I5V
ygeK	2992918	8	G/A	A3V
xdhB	3000749	114	G/A	Q38Q
xdhB	3001503	868	A/G	K290E
ygeV	3003178	631	T/C	T211A
ygeW	3005301	1018	T/C	C340R
ygeX	3005857	326	T/C	I109T
ygeX	3006568	1037	T/C	V346A
ygeY	3007489	704	G/A	G235D
hyuA	3008140	91	T/C	L31L
yqeB	3010814	1448	T/C	D483G
yqeB	3011454	808	G/A	L270L
yqeB	3011487	775	T/C	M259V
yqeB	3011615	647	G/A	A216V
xdhD	3020733	1396	C/T	R466C
xdhD	3021735	2398	A/G	T800A
ygfT	3027042	1912	C/T	A638T
ygfU	3029856	468	T/C	T156T
ygfU	3030506	1118	T/C	V373A
prfB	3033968	261	C/T	Q87Q
recJ	3034756	1373	T/C	D458G
recJ	3035432	697	T/C	T233A
ygfX	3038486	360	T/C	E120E
gcvP	3044393	2671	G/A	P891S
gcvP	3044710	2354	G/A	A785V
gcvP	3045979	1085	T/C	N362S
gcvP	3046286	778	T/C	K260E

Table S3: Intragenic single nucleotide variations in strain THY2.

Gene	Chromosomal position	Gene position	Mutation	Amino acid change
pepP	3052335	528	G/A	F176F
ygfB	3053070	397	C/T	A133T
ygfA	3054378	116	T/C	V39A
rpiA	3057249	99	T/C	A33A
argK	3061240	232	T/C	L78L
ygfH	3063383	560	T/C	V187A
ygfI	3064430	766	T/C	T256A
ygfI	3064843	353	T/C	Y118C
mscS	3067474	356	G/A	A119V
fbaA	3068560	707	A/G	V236A
epd	3071531	183	T/C	V61V
yggD	3073126	92	A/G	L31P
endA	3088486	118	G/A	A40T
gshB	3090115	216	A/G	E72E
yggS	3093191	72	T/C	R24R
yggW	3096185	897	G/A	Q299Q
yggH	3100439	436	A/G	W146R
mutY	3101391	357	T/C	R119R
mltC	3102603	149	A/G	N50S
mltC	3102949	495	C/T	S165S
mltC	3103406	952	G/A	A318T
nupG	3104286	551	T/C	V184A
glcB	3121812	16	T/C	T6A
glcD	3125958	86	A/G	V29A
glcC	3126305	12	A/G	E4E
yghO	3127679	559	A/G	W187R
yghO	3127695	543	G/A	G181G
yghQ	3129723	708	T/C	K236K
gss	3135318	1227	T/C	E409E
gss	3136123	422	T/C	N141S
hybC	3140269	743	T/C	D248G
hybB	3141910	277	A/G	L93L
yqhG	3156035	364	A/G	T122A
ygiQ	3157413	1756	G/A	L586L
ygiQ	3158480	689	G/A	A230V
sufI	3160630	62	A/G	V21A
ygiS	3164597	1144	A/G	F382L
qseB	3168333	484	A/G	M162V
qseC	3168542	37	C/T	L13L
tolC	3176260	124	T/C	S42P
tolC	3177372	1236	T/C	R412R
ygiA	3177649	32	A/G	N11S
yqiK	3191674	789	T/C	R263R
rfaE	3193633	1143	T/C	K381K
glnE	3197372	292	T/C	M98V
bacA	3201455	699	G/A	T233T
ttdA	3204653	169	T/C	F57L

Table S3: Intragenic single nucleotide variations in strain THY2.

Gene	Chromosomal position	Gene position	Mutation	Amino acid change
ttdA	3205129	645	G/A	L215L
ygjE	3207271	1226	T/C	I409T
dnaG	3210502	1374	G/A	L458L
rpoD	3211384	316	G/A	G106S
rpoD	3211451	383	A/G	N128S
rpoD	3212741	1673	T/C	V558A
ygjF	3213063	433	T/C	S145G
ygjH	3213850	664	T/C	T222A
aer	3216827	272	T/C	D91G
ygjG	3217823	335	T/C	V112A
ygjG	3218053	565	T/C	F189L
ebgA	3220965	311	T/C	V104A
ebgA	3221321	667	G/A	A223T
ebgA	3221598	944	T/C	V315A
ebgA	3222233	1579	T/C	Y527H
ebgA	3223530	2876	A/G	D959G
ygjJ	3226531	709	G/A	E237K
fadH	3231542	1856	A/G	D619G
ygjO	3233583	315	T/C	Q105Q
ygjP	3234231	250	C/T	H84Y
alx	3237543	942	G/A	R314R
uxaC	3241799	965	T/C	N322S
yhaJ	3251948	289	T/C	T97A
yhaO	3255568	402	A/G	S134S
tdcC	3262861	179	T/C	Y60C
sohA	3275278	255	T/C	R85R
agaI	3284615	324	G/A	V108V
yraJ	3288023	1188	T/C	G396G
yhbT	3298987	312	T/C	K104K
yhbV	3301029	519	G/A	P173P
yhbW	3301571	102	T/C	R34R
yhbW	3302028	559	T/C	F187L
yhbW	3302064	595	T/C	F199L
infB	3312229	1808	T/C	Y603C
infB	3313378	659	T/A	E220V
infB	3313419	618	T/C	A206A
yhbX	3318513	1123	C/T	A375T
yhbX	3319480	156	T/C	K52K
glmM	3321065	1028	A/G	V343A
folP	3322122	812	G/A	A271V
ftsH	3324888	70	G/A	P24S
rrmJ	3325328	359	A/G	V120A
obgE	3329616	161	T/C	N54S
yrbC	3335692	222	A/G	G74G
yrbD	3336157	327	T/C	E109E
yrbF	3337489	599	A/G	V200A
yrbK	3341062	205	T/C	Y69H

Table S3: Intragenic single nucleotide variations in strain THY2.

Gene	Chromosomal position	Gene position	Mutation	Amino acid change
yh bH	3344425	231	A/G	L77L
elbB	3348000	482	T/C	E161G
arcB	3349502	1546	A/G	F516L
arcB	3349656	1392	T/C	K464K
gltB	3355362	2709	C/T	A903A
yhcG	3366600	752	C/T	A251V
yhcG	3366606	758	T/C	V253A
nanK	3367513	860	T/C	Q287R
nanK	3367804	569	T/C	E190G
dcuD	3373834	944	A/G	N315S
rpsI	3376050	180	T/C	K60K
yhcM	3377653	367	T/C	T123A
mdh	3381881	410	A/G	V137A
yhcN	3383666	107	C/T	A36V
aaeA	3386424	725	T/C	D242G
yhdP	3392921	1360	T/C	T454A
yhdP	3393710	571	T/C	T191A
rng	3394436	1382	G/A	A461V
mreC	3396950	1051	T/C	T351A
yhdH	3402051	546	T/C	S182S
accC	3404320	382	G/A	V128I
panF	3405824	196	C/T	P66S
panF	3406882	1254	A/G	E418E
dusB	3409238	937	T/C	L313L
envR	3410984	504	A/G	G168G
acrE	3412713	828	T/C	R276R
acrF	3413275	221	A/G	N74S
acrF	3413449	395	C/T	S132F
acrF	3414822	1768	T/C	L590L
acrF	3415153	2099	A/G	Q700R
acrF	3415578	2524	T/C	L842L
acrF	3416144	3090	T/C	R1030R
smf	3430647	936	C/T	E312E
fnt	3432396	161	T/C	V54A
fnt	3432996	761	T/A	I254N
rsmB	3434170	942	A/G	Q314Q
trkA	3435900	1361	G/A	S454N
mseL	3436449	404	G/A	R135H
rplQ	3437830	192	A/G	R64R
rpoA	3438314	738	T/C	K246K
secY	3441295	825	G/A	I275I
pioO	3451820	130	T/C	M44V
gspA	3452225	1196	T/C	E399G
gspA	3452921	500	A/G	L167P
gspA	3453218	203	T/C	N68S
gspC	3454023	424	T/C	F142L
gspH	3459508	19	T/C	F7L

Table S3: Intragenic single nucleotide variations in strain THY2.

Gene	Chromosomal position	Gene position	Mutation	Amino acid change
gspK	3461188	243	G/A	Q81Q
gspL	3462357	414	C/T	G138G
rpsG	3471682	422	A/G	V141A
rpsG	3472041	63	T/C	E21E
tusD	3473400	341	G/A	A114V
kefG	3478833	351	A/G	S117S
yheS	3481216	1906	A/G	S636G
yheT	3481960	737	T/C	I246T
tsgA	3490627	38	G/A	S13N
tsgA	3491217	628	T/C	F210L
nirD	3494823	251	G/A	C84Y
nirC	3495133	109	T/C	Y37H
frlA	3498877	946	T/C	F316L
frlB	3499629	340	C/T	L114L
yhfS	3503038	1005	A/G	H335H
yhfS	3503205	838	C/T	V280M
yhfU	3505599	125	G/A	S42F
yhfW	3507369	467	G/A	A156V
gph	3512008	404	T/C	N135S
yrfA	3518970	72	T/C	K24K
mrcA	3521936	1044	G/A	P348P
igaA	3526210	1720	G/A	A574T
hslO	3528500	705	A/G	E235E
yhgE	3528885	1577	T/C	D526G
pck	3532018	1179	T/C	G393G
bioH	3542859	8	T/C	N3S
gntT	3545542	962	T/C	V321A
malQ	3547151	942	G/A	G314G
malP	3548927	1569	G/A	I523I
malP	3550236	260	T/C	D87G
malT	3553597	2491	A/G	I831V
glgP	3563005	1600	G/A	Q534*
glgA	3566019	38	A/G	L13P
glgX	3568603	740	G/A	A247V
glgX	3568716	627	A/G	R209R
asd	3572552	350	T/C	D117G
yhgN	3573526	433	G/A	A145T
gntU	3573883	1202	T/C	N401S
ryhB	3578953	87	A/-	
yhhZ	3580047	162	T/C	G54G
ggt	3584392	455	G/A	A152V
ggt	3584725	122	A/G	F41S
ugpC	3586145	1059	T/C	G353G
livF	3590990	471	A/G	R157R
livG	3591483	747	A/G	R249R
livH	3594151	276	T/C	E92E
yhhK	3596196	190	G/A	D64N

Table S3: Intragenic single nucleotide variations in strain THY2.

Gene	Chromosomal position	Gene position	Mutation	Amino acid change
ftsE	3600493	278	T/C	D93G
ftsY	3601667	600	T/C	K200K
yhhN	3603870	97	T/C	L33L
zntA	3605406	933	T/C	R311R
yhhQ	3607708	469	G/A	A157T
yhhT	3610755	868	A/G	M290V
acpT	3611176	185	C/T	A62V
nikD	3615446	409	T/C	Y137H
yhhI	3623484	1084	G/A	A362T
yhhJ	3624468	359	T/C	N120S
yhiL	3631057	211	T/C	T71A
c0362	3637248	54	A/G	
yhiP	3639398	514	T/C	Y172H
yhiQ	3641047	109	A/G	L37L
slp	3652265	282	G/A	L94L
mdtF	3659823	1387	A/G	T463A
mdtF	3661520	3084	A/G	E1028E
yhjC	3671141	777	T/C	G259G
yhjD	3671416	32	T/C	I11T
yhjE	3673598	790	T/C	F264L
yhjG	3676140	249	T/C	V83V
yhjJ	3679436	528	G/A	G176G
dctA	3680955	516	T/C	Q172Q
yhjK	3682539	1064	T/C	Y355C
bcsC	3684500	2697	A/G	S899S
bcsA	3692149	1111	G/A	Q371*
bcsA	3692844	416	T/C	E139G
bcsA	3693219	41	T/C	N14S
bcsG	3697194	958	C/T	H320Y
dppC	3702167	618	C/T	Q206Q
dppB	3702997	817	T/C	T273A
dppA	3704702	1027	A/G	W343R
rtt	3706522	105	T/C	
rtt	3706536	91	T/C	
bisC	3713386	978	T/C	A326A
yiaD	3714693	124	G/A	G42S
yiaF	3716908	160	T/C	T54A
yiaG	3717538	38	G/A	S13N
hokA	3718537	87	A/G	C29C
insK	3719473	253	A/G	T85A
glyS	3720417	2004	A/G	R668R
glyS	3721369	1052	G/A	T351I
xylB	3727371	24	G/A	G8G
yiaK	3740799	44	T/C	V15A
yiaN	3743562	736	T/C	F246L
lyxK	3745681	575	A/G	D192G
yiaU	3750772	758	A/G	D253G

Table S3: Intragenic single nucleotide variations in strain THY2.

Gene	Chromosomal position	Gene position	Mutation	Amino acid change
selB	3756190	1695	A/G	G565G
mtlA	3770600	297	T/C	G99G
gpsA	3781593	92	A/G	V31A
waaK	3796682	654	T/-	
waaK	3796773	563	T/C	N188S
waaK	3796963	373	T/C	T125A
waaZ	3797634	586	T/C	T196A
waaZ	3797696	524	G/A	A175V
waaY	3798776	213	A/G	R71R
recG	3824828	1596	A/G	K532K
yicH	3828825	346	C/T	P116S
yicO	3840565	1248	T/C	K416K
yicO	3840715	1098	A/G	V366V
ade	3843347	1361	G/A	C454Y
yidK	3856947	1193	T/C	N398S
yidK	3857110	1030	T/C	T344A
ibpB	3864830	91	T/C	S31G
yidQ	3865850	100	A/G	T34A
dgoD	3870373	649	C/T	A217T
dgoD	3870668	354	A/G	S118S
dgoA	3871463	173	T/C	D58G
yidB	3875387	102	C/T	V34V
yieF	3893176	502	A/G	T168A
cbrC	3896575	531	T/C	G177G
yieK	3897142	275	C/T	G92E
bglF	3902835	786	C/T	L262L
pstC	3907797	625	A/G	C209R
pstC	3908396	26	T/C	N9S
pstS	3908860	689	G/A	A230V
glmS	3911509	183	T/C	E61E
glmU	3912007	1217	T/C	D406G
atpI	3920354	110	C/T	G37D
atpI	3920426	38	T/C	K13R
gidA	3921957	1700	T/C	N567S
gidA	3923113	544	G/A	P182S
gidA	3923346	311	T/C	Y104C
rbsA	3932549	749	A/G	D250G
rbsA	3933254	1454	A/G	E485G
rbsC	3933780	470	A/G	N157S
rbsB	3934497	197	A/G	N66S
rbsK	3935992	676	A/G	S226G
rbsR	3936754	505	A/G	T169A
hsrA	3938103	533	C/T	G178D
yifB	3946952	1071	G/A	S357S
ilvC	3956890	898	T/C	F300L
gpp	3961191	1062	C/T	L354L
gpp	3961282	971	A/G	I324T

Table S3: Intragenic single nucleotide variations in strain THY2.

Gene	Chromosomal position	Gene position	Mutation	Amino acid change
gpp	3961784	469	A/G	L157L
wzzE	3967266	213	A/G	G71G
rffD	3970191	909	G/A	V303V
rffC	3972760	271	A/G	T91T
rffA	3974281	1113	G/A	L371L
yifK	3978933	24	A/G	L8L
hemD	3987148	704	T/C	N235S
cyaA	3989216	41	C/T	A14V
cyaA	3991240	2065	T/C	F689L
yigA	3993817	212	A/G	N71S
yigE	3998988	92	A/G	V31A
pldB	4007714	522	A/T	S174S
yigM	4009844	746	A/G	N249S
rmuC	4016050	695	A/C	E232A
tatD	4022216	640	C/T	P214S
trkH	4031854	687	T/C	S229S
mobB	4039426	16	C/T	A6T
yihF	4042288	67	T/C	Y23H
yihF	4042551	330	C/T	V110V
yihF	4043594	1373	A/G	N458S
csrC	4049143	85	A/G	
ompL	4061902	417	T/C	E139E
yihP	4063875	1364	A/G	V455A
yihP	4065223	16	T/C	T6A
yihS	4069674	106	A/G	L36L
yihW	4072782	91	G/A	A31T
yihW	4072943	252	A/-	
fdoG	4081045	2801	G/A	A934V
fdoG	4081974	1872	A/G	G624G
fdoG	4082309	1537	A/G	F513L
rhaS	4096244	486	G/A	L162L
sodA	4099404	572	A/G	N191S
kdgT	4099745	33	G/A	P11P
kdgT	4099893	181	T/C	S61P
yiiM	4101277	433	T/C	L145L
cpxA	4102455	544	A/G	L182L
yiiQ	4109973	265	A/G	Y89H
ftsN	4121330	33	T/C	Q11Q
cytR	4122403	77	A/G	V26A
metL	4127970	113	A/G	Y38C
metL	4129140	1283	A/G	D428G
metF	4130854	216	C/T	S72S
katG	4132515	658	A/G	T220A
katG	4133110	1253	C/T	P418L
yijE	4134858	728	C/T	P243L
pflC	4144418	138	C/T	G46G
yijP	4146897	1392	T/C	E464E

Table S3: Intragenic single nucleotide variations in strain THY2.

Gene	Chromosomal position	Gene position	Mutation	Amino acid change
yijP	4147590	699	A/G	G233G
yijP	4147981	308	A/G	V103A
argE	4151963	908	G/A	P303L
oxyR	4156785	273	A/G	G91G
oxyR	4157180	668	G/A	S223N
yijD	4159820	27	T/C	G9G
rpoB	4180739	1472	A/G	D491G
rpoB	4182653	3386	A/G	N1129S
rpoC	4183441	69	T/C	A23A
rpoC	4184610	1238	A/G	D413G
rpoC	4187273	3901	A/G	T1301A
sroH	4188493	18	A/G	
thiE	4192219	9	C/T	Q3Q
thiC	4192891	1232	G/A	A411V
nfi	4196852	40	G/A	A14T
hupA	4198381	78	G/A	E26E
zraS	4200658	710	A/G	K237R
purD	4203753	202	T/C	T68A
purH	4204012	1544	T/C	H515R
aceK	4217607	989	G/A	G330D
arpA	4219887	624	A/G	R208R
metH	4225223	3373	C/T	L1125L
yjbH	4236901	1245	C/T	G415G
malE	4244181	262	A/G	W88R
malE	4244304	139	C/T	A47T
lamB	4246498	505	A/G	T169A
malM	4248174	598	A/G	T200A
ubiA	4251736	698	A/G	N233S
yjbM	4258905	284	T/C	V95A
soxR	4275885	394	T/C	L132L
yjcD	4277394	893	C/T	A298V
actP	4281860	1066	A/G	F356L
yjcP	4298560	494	A/G	V165A
yjcP	4299013	41	A/G	I14T
yjcS	4304221	400	A/G	S134P
alsA	4307580	1424	A/G	V475A
alsA	4307872	1132	C/T	G378S
alsB	4309882	184	A/G	S62P
rpiB	4311610	238	G/A	A80T
phnH	4319220	51	A/G	S17S
phnE	4320794	41	T/C	K14R
phnE	4320794	511	T/C	S171G
yjdA	4325834	677	A/G	E226G
melB	4341489	86	A/G	Y29C
fumB	4344236	1114	C/T	A372T
fumB	4344507	843	C/T	Q281Q
dcuS	4348924	762	C/T	L254L

Table S3: Intragenic single nucleotide variations in strain THY2.

Gene	Chromosomal position	Gene position	Mutation	Amino acid change
cadB	4357114	941	T/C	D314G
yjeH	4367309	1127	A/G	V376A
yjeH	4367351	1085	C/T	G362D
yjeH	4367852	584	C/T	W195*
sugE	4374907	10	A/G	I4V
blc	4375616	130	A/G	Y44H
frdB	4378322	219	C/T	V73V
yjeM	4382793	932	G/A	W311*
yjeP	4384309	3085	A/G	F1029L
yjeP	4386334	1060	G/A	L354F
psd	4388117	267	G/A	A89A
rsgA	4388480	1053	T/C	*351W
rsgA	4388484	1049	T/C	D350G
orn	4389933	307	C/T	P103S
yjeS	4391450	641	A/G	V214A
yjeS	4391843	248	A/G	L83P
yjeS	4392010	81	A/G	G27G
mutL	4395859	425	G/A	G142E
mutL	4396826	1392	A/G	E464E
hflX	4398993	299	T/C	V100A
hflX	4399507	813	A/G	L271L
hflK	4400423	363	T/C	G121G
hflK	4400743	683	A/G	D228G
yjeT	4402595	187	A/G	T63A
purA	4403119	410	C/T	A137V
yjfJ	4409031	456	A/G	Q152Q
yjfJ	4409168	593	A/G	D198G
yjfC	4412070	1020	A/G	Q340Q
aidB	4413736	1439	A/G	E480G
yjfO	4414599	195	A/G	G65G
ulaB	4419491	76	T/C	F26L
ulaD	4420746	538	T/C	F180L
ytfL	4438741	498	T/C	K166K
ytfN	4443021	887	A/G	D296G
ytfN	4443704	1570	C/T	P524S
fbp	4453235	398	C/T	R133H
fbp	4453373	260	G/A	A87V
pmbA	4456319	338	A/G	D113G
nrdD	4459093	1591	A/G	W531R
treB	4463428	776	A/G	L259P
mgtA	4467298	1651	A/G	T551A
mgtA	4467966	2319	A/G	G773G
pyrB	4470104	315	C/T	M105I
argI	4475702	633	C/T	G211G
argI	4476161	174	A/G	R58R
valS	4479925	1936	A/G	W646R
valS	4480963	898	T/C	I300V

Table S3: Intragenic single nucleotide variations in strain THY2.

Gene	Chromosomal position	Gene position	Mutation	Amino acid change
idnT	4489489	1060	C/T	A354T
idnT	4490346	203	C/T	G68E
idnO	4490616	759	A/G	A253A
idnO	4490645	730	A/G	L244L
idnD	4491815	615	A/G	R205R
yjgB	4493511	722	A/G	V241A
intB	4495294	522	A/G	Q174Q
yjhB	4502360	280	A/G	T94A
yjhC	4503701	392	A/G	K131R
yjhC	4504301	992	A/G	Y331C
yjhW	4507602	29	A/G	N10S
sgeC	4527311	956	A/G	I319T
sgeX	4528987	688	G/A	L230L
sgeX	4529308	367	C/T	A123T
yjhP	4530744	463	C/T	A155T
yjhP	4531085	122	A/G	L41P
yjhS	4535306	312	C/T	L104L
yjhS	4535403	215	A/G	V72A
fimE	4540325	266	A/G	E89G
gntP	4548487	833	A/G	V278A
gntP	4548562	758	G/A	T253I
gntP	4549317	3	C/T	M1I
yjiD	4555156	141	C/T	G47G
yjiM	4563755	119	C/T	C40Y
yjiQ	4567845	465	A/G	E155E
yjiR	4568272	1326	C/T	L442L
yjiR	4568995	603	A/G	C201C
yjiR	4569024	574	C/T	G192S
yjiV	4572671	514	G/A	D172N
yjiV	4574319	2162	A/G	D721G
yjiV	4574812	2655	A/G	E885E
mcrC	4575065	917	A/G	V306A
hsdM	4580329	743	T/C	N248S
hsdR	4583123	1716	T/C	K572K
hsdR	4584295	544	T/C	T182A
mrr	4585681	710	G/A	G237D
yjiA	4586421	468	C/T	L156L
yjiY	4587859	1444	G/A	P482S
yjiY	4588279	1024	C/T	G342S
yjiA	4598023	190	T/C	N64D
yjiA	4598063	150	A/G	G50G
rsmC	4605503	221	A/G	V74A
rimI	4606264	57	A/G	E19E
yjiG	4607250	582	G/A	W194*
prfC	4607968	532	C/T	L178L
prfC	4608623	1187	A/G	E396G
osmY	4609498	80	A/G	Y27C

Table S3: Intragenic single nucleotide variations in strain THY2.

Gene	Chromosomal position	Gene position	Mutation	Amino acid change
nadR	4625476	139	A/G	K47E
slt	4629137	382	A/G	T128A
rob	4633060	274	A/G	F92L
creC	4634842	124	T/C	L42L
creC	4634900	182	A/G	D61G
creC	4635912	1194	A/G	E398E
yjjY	4638474	50	A/G	N17S

Table S4: Intergenic single nucleotide variations in strain THY2. The intergenic region, the chromosomal position and nature of the mutation are listed.

Intergenic region	Chromosomal position	Mutation
folA - apaH	50358	G/A
imp - djlA	57348	T/C
leuO - ilvI	85628	C/T
fruR - mraZ	89551	G/A
ppdD - nadC	117550	C/T
yafT - c0067	238452	C/T
lpcA - yafJ	244323	A/G
pepD - gpt	255755	C/T
insI - insO	271014	A/G
yagM - yagN	294203	T/C
yagT - yagU	302205	C/T
yagU - ykgJ	302943	A/G
yagY - ecpA	309305	A/G
ykgG - ykgH	323755	C/T
betT - yahA	331116	A/G
betT - yahA	331136	C/T
betT - yahA	331270	G/A
betT - yahA	331568	A/G
prpR - prpB	347896	A/G
frmR - yaiO	379246	G/-
psiF - yaiC	402850	C/T
proY - malZ	421628	C/T
ylaC - maa	478511	A/G
priC - ybaN	490075	G/T
aes - gsk	499304	T/C
ybbD - ylbG	528718	A/G
folD - sfmA	557007	G/A
ybcQ - insH	573679	G/A
nfrB - cusS	592543	C/T
entF - fepE	617474	C/T
cstA - ybdD	631384	A/G
dsbG - ahpC	638065	A/G
citB - dcuC	653801	A/G
ybeB - phpB	668510	A/-
metT - asnB	696522	T/C
glnS - ybfM	707141	T/C
pgm - ybfP	714439	A/-
galT - galE	790254	G/A
ybiC - ybiJ	836767	A/G

Table S4: Intergenic single nucleotide variations in strain THY2.

Intergenic region	Chromosomal position	Mutation
rhtA - ompX	849480	A/G
ybjT - ltaE	907507	T/C
serW - infA	925307	A/G
infA - aat	925947	A/G
pncB - pepN	989736	T/C
serT - hyaA	1030949	C/T
pgaA - ycdT	1091946	A/G
ycfQ - ycfR	1168063	T/C
ymgG - ymgH	1222211	A/G
ycgB - dadA	1236522	A/G
dhaK - dhaR	1250102	C/T
dhaK - dhaR	1250120	T/C
chaB - chaC	1271664	C/T
ychJ - rssA	1288373	A/G
ychE - oppA	1298940	A/G
yciN - topA	1328870	T/C
yciX - acnA	1333732	A/G
ycjF - tyrR	1384614	A/G
yneI - yneJ	1612748	T/C
ydeH - ydeI	1622095	G/A
flxA - ydfW	1644975	A/G
ynfA - ynfB	1653809	A/G
pntA - ydgH	1676153	A/G
uidA - uidR	1694411	A/G
hdhA - mall	1696080	A/G
ydgT - ydgK	1703253	C/T
slyA - ydhI	1719033	T/C
ydhC - cfa	1739415	A/G
yeaP - yeaQ	1876825	T/C
yeaR - yeaS	1877981	C/T
yeaZ - yoaA	1889295	T/C
yobD - yebN	1903414	T/C
edd - zwf	1932635	T/C
zwf - yebK	1934603	T/C
torY - cutC	1956254	A/G
insA - yecG	1977770	A/G
yedQ - yodC	2026056	A/G
yedW - yedX	2036874	T/C
cobU - insH	2064075	T/C
insH - yoeA	2065923	T/C
wcaA - wzc	2131428	T/C
yegI - yegJ	2149054	A/G
yegJ - yegK	2149721	C/T

Table S4: Intergenic single nucleotide variations in strain THY2.

Intergenic region	Chromosomal position	Mutation
yehE - mrp	2190852	G/A
yojL - ompC	2309594	C/T
argT - ubiX	2426033	T/C
dedA - truA	2432818	G/A
usg - pdxB	2434714	C/T
fadL - yfdF	2460911	C/-
yfdY - ddg	2493341	A/G
yfeA - alaX	2516007	A/G
yffN - yffO	2560112	T/-
yfgF - yfgG	2627256	T/C
hisS - ispG	2638599	T/C
iscR - yfhQ	2660458	T/C
iscR - yfhQ	2660561	T/C
aroF - yfiL	2739363	T/C
ypjB - ileY	2783604	A/G
ygaP - stpA	2796082	G/A
stpA - ygaW	2796857	C/T
yqaB - argQ	2815528	T/C
mltB - srlA	2823733	A/G
norR - norV	2830473	A/G
iap - ygbF	2875743	G/A
ygcE - ygcF	2902426	A/G
ptr - recC	2957079	C/T
yqeF - yqeG	2983754	G/A
yqeG - yqeH	2985218	T/C
ygeI - pbl	2992060	A/G
ygfY - ygfZ	3039329	T/C
gcvP - gcvH	3047106	T/C
fbaA - pgk	3069347	T/C
yggW - yggM	3096538	G/-
yqhA - yghA	3147609	A/G
ygiN - parE	3171517	T/C
ygiE - ribB	3181361	T/C
yqiI - glgS	3189756	T/C
rygD - rfaE	3193129	T/C
rygD - rfaE	3193133	-/C
ygiF - htrG	3199205	T/C
rpsU - dnaG	3209123	G/A
yhaC - c0362	3267687	G/A
garL - garP	3271589	C/T
ispB - sfsB	3332707	C/T
yrbF - yrbG	3338188	C/T
yhcC - gltB	3352525	T/C

Table S4: Intergenic single nucleotide variations in strain THY2.

Intergenic region	Chromosomal position	Mutation
nanT - nanA	3370617	C/T
yhdN - rplQ	3437544	G/A
nudE - igaA	3524461	G/A
yhiJ - yhiL	3630800	G/A
gor - arsR	3646250	T/C
gadX - gadA	3663956	T/C
yhjB - yhjC	3670250	G/A
viaW - aldB	3752573	T/C
rph - yicC	3814634	C/T
yicC - dinD	3815779	G/A
selC - setC	3834357	T/C
yicS - yicM	3838558	T/C
dnaA - rpmH	3882286	T/C
glmU - atpC	3913418	G/A
gidA - mioC	3923973	A/G
ilvL - ilvG	3948491	G/T
hemC - cyaA	3989158	C/T
rrfA - mobB	4038741	A/G
hemN - glnG	4051803	G/A
ppc - argE	4151125	A/G
argH - oxyS	4156249	A/G
sroH - thiH	4188748	A/G
thiC - rsd	4194287	C/T
hupA - yjaH	4198583	G/A
lysC - pgi	4231496	A/G
xyle - malG	4240282	A/G
yjbJ - zur	4257503	A/G
ryjA - yjcD	4276501	T/C
phnA - yjdA	4325100	A/G
dcuB - dcuR	4347326	A/G
lysU - yjdL	4352826	A/G
cadC - pheU	4360142	A/G
yjeH - groS	4368439	T/C
groL - yjeI	4370817	A/G
frdA - poxA	4380369	A/G
ytfJ - ytfK	4437489	-/A
msrA - ytfM	4440243	A/G
mgtA - yjgF	4468392	A/G
yjgX - yjgZ	4498951	T/C
yjgZ - insG	4500006	A/G
yjhV - fecE	4508188	T/C
fecI - insA	4516405	G/A
yjhI - sgcR	4523890	T/C

Table S4: Intergenic single nucleotide variations in strain THY2.

Intergenic region	Chromosomal position	Mutation
yjhI - sgcR	4523959	A/G
yjhA - fimB	4538276	A/G
yjiC - yjiD	4554792	T/-
yjiK - hgdC	4561785	A/G
mdoB - yjjA	4597576	T/C
yjjG - prfC	4607349	G/A
nadR - yjjK	4626716	T/C
yjjY - yjtD	4638609	G/A

Table S5: Intragenic single nucleotide variations in strain THY4. The gene affected, chromosomal position, position in the gene and the nature of the mutation are listed together with its consequence on the encoded amino acid. * indicates a stop codon; blanks in the "Amino acid change" column indicate either frameshift mutations or mutations in non translated RNAs.

Gene	Chromosomal Position	Gene Position	Mutation	Amino acid change
leuB	81253	706	C/T	D236N
lpd	128153	242	C/T	T81I
yagK	292010	163	A/G	F55L
cusF	596418	65	C/T	A22V
fes	612991	954	C/G	R318R
moaA	817143	877	C/T	Q293*
ybhS	826368	108	C/T	P36P
yliE	872474	273	G/A	M91I
ybjL	888381	662	C/T	G221D
ymcC	1048427	129	C/T	Q43Q
rne	1141650	1941	C/T	Q647Q
ycfH	1156253	254	C/T	A85V
ndh	1165682	375	C/T	T125T
ycgH	1221429	1001	C/A	T334K
ymgH	1222893	206	A/C	E69A
ymgH	1222894	207	A/C	E69D
narI	1285282	211	C/T	L71L
tdk	1293210	461	T/C	V154A
cls	1306245	425	C/T	R142H
trpB	1316161	279	C/T	G93G
pyrF	1340506	562	A/G	T188A
yciW	1347167	965	G/A	A322V
ycjT	1377362	1455	C/T	S485S
ycjX	1382812	672	T/G	G224G
abgA	1401818	772	G/T	Q258K
azoR	1480726	159	G/C	G53G
yneK	1614286	500	T/C	V167A
cspF	1639950	72	C/A	S24S
ynfK	1664784	460	G/A	L154L
ydgR	1711355	563	T/C	V188A
anmK	1717161	466	G/A	L156L
lhr	1728023	913	G/A	A305T
ydhB	1736966	857	T/C	E286G
ydhV	1750443	1412	A/G	V471A
ydiU	1788088	1181	G/A	A394V
sppA	1847340	480	T/C	Y160Y
yeaV	1881276	65	T/C	L22P
adrB	1896761	311	A/G	D104G

Table S5: Intragenic single nucleotide variations in strain THY4.

Gene	Chromosomal Position	Gene Position	Mutation	Amino acid change
zwf	1934188	151	C/T	A51T
fliH	2014558	667	G/A	A223T
yedS	2032124	50	G/A	G17D
gatD	2170565	333	C/G	S111S
yehB	2187896	1037	C/A	R346L
molR	2195653	222	G/A	L74L
yehM	2204600	884	C/G	A295G
mglA	2236771	541	T/C	T181A
yeiB	2239950	1040	G/C	A347G
cirA	2244651	141	T/G	A47A
ypdD	2502210	298	G/A	L100L
yfeC	2516527	39	C/T	S13S
yffO	2560141	9	C/G	G3G
is128	2651594	58	C/T	
lepB	2702522	810	G/T	F270L
ypjB	2782979	55	T/C	S19G
fucP	2932546	290	G/A	G97E
ygfK	3014875	794	T/C	V265A
yliK	3060570	1699	T/C	F567L
pgk	3069858	787	A/G	F263L
yqiG	3187393	1972	G/A	G658S
ygjF	3213276	220	T/C	T74A
infB	3313701	336	C/T	A112A
infB	3313704	333	T/C	E111E
infB	3313710	327	C/A	Q109H
infB	3313711	326	T/C	Q109R
infB	3313713	324	C/G	A108A
arcB	3350694	354	T/C	E118E
fnt	3432996	761	T/A	I254N
yhhZ	3580835	950	T/C	V317A
yhjG	3675283	1106	G/C	A369G
yibA	3765063	704	A/-	
rph	3814394	179	G/C	T60S
hdfR	3945846	145	-/G	
ilvA	3953648	295	G/A	A99T
fabR	4159171	82	G/A	E28K
rpoC	4186304	2932	C/T	R978C
yjcF	4280224	875	A/G	V292A
yjcF	4280811	288	A/G	A96A
alsA	4308801	203	C/T	S68N
yjdL	4353079	1356	T/C	G452G
cadC	4359595	363	G/A	P121P
ulaE	4421251	383	A/G	N128S
ytfQ	4448806	822	T/G	G274G
treC	4461443	1290	G/C	G430G
pepA	4483913	62	C/T	G21D
nadR	4626247	910	A/G	K304E

Table S6: Intergenic single nucleotide variations in strain THY4. The intergenic region, the chromosomal position and nature of the mutation are listed.

Intergenic region	Chromosomal position	Mutation
leuL - leuO	83868	G/A
frr - dxr	193480	C/G
alaV - rrlH	225742	A/G
ykgM - eaeH	312014	T/C
lacZ - lacI	365547	A/G
priC - ybaN	490075	G/T
rhsD - ybbC	526784	T/C
ybgF - lysT	779673	T/C
ompF - asnS	986237	A/C
serX - ycdW	1096912	T/C
dhaK - dhaR	1250102	C/T
dhaK - dhaR	1250169	C/T
hns - tdk	1292305	C/T
insC - insI	1467364	G/A
uxaB - yneF	1608716	T/C
ynfD - ynfE	1656069	A/G
manA - ydgA	1687828	C/T
anmK - slyB	1717813	A/G
pps - ydiA	1785347	G/A
chbB - osmE	1819749	C/T
ynjF - nudG	1839462	G/A
cysT - glyW	1990023	G/A
yehA - yehB	2186440	A/G
yeiA - mglC	2234727	T/C
argW - intS	2464417	G/A
cysP - ucpA	2541590	C/T
aroF - yfiL	2739316	C/T
ypjA - pinH	2781026	T/C
ileY - ygaR	2784119	C/T
argQ - argZ	2815981	G/C
argY - argV	2816396	G/C
ygeV - ygeW	3004167	G/A
cmtB - tktA	3077500	A/G
yggN - yggL	3099796	C/T
insH - slp	3651853	T/C
insH - slp	3651884	A/G
dgoR - yidX	3873290	T/C
glmU - atpC	3913463	A/G
rrsA - ileT	4035156	A/G

Table S6: Intergenic single nucleotide variations in strain
THY4

Intergenic region	Chromosomal position	Mutation
yihG - polA	4044868	A/G
rplL - rpoB	4178983	C/T
sroH - thiH	4188560	T/G
yjhA - fimB	4537959	T/C

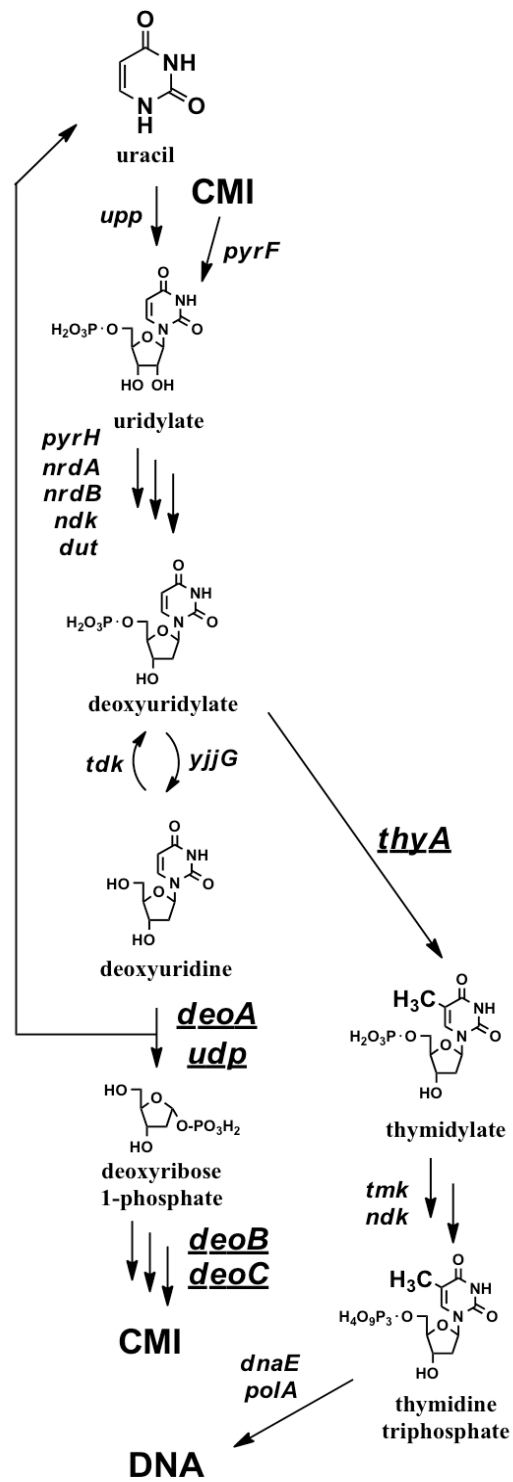


Figure S1a)

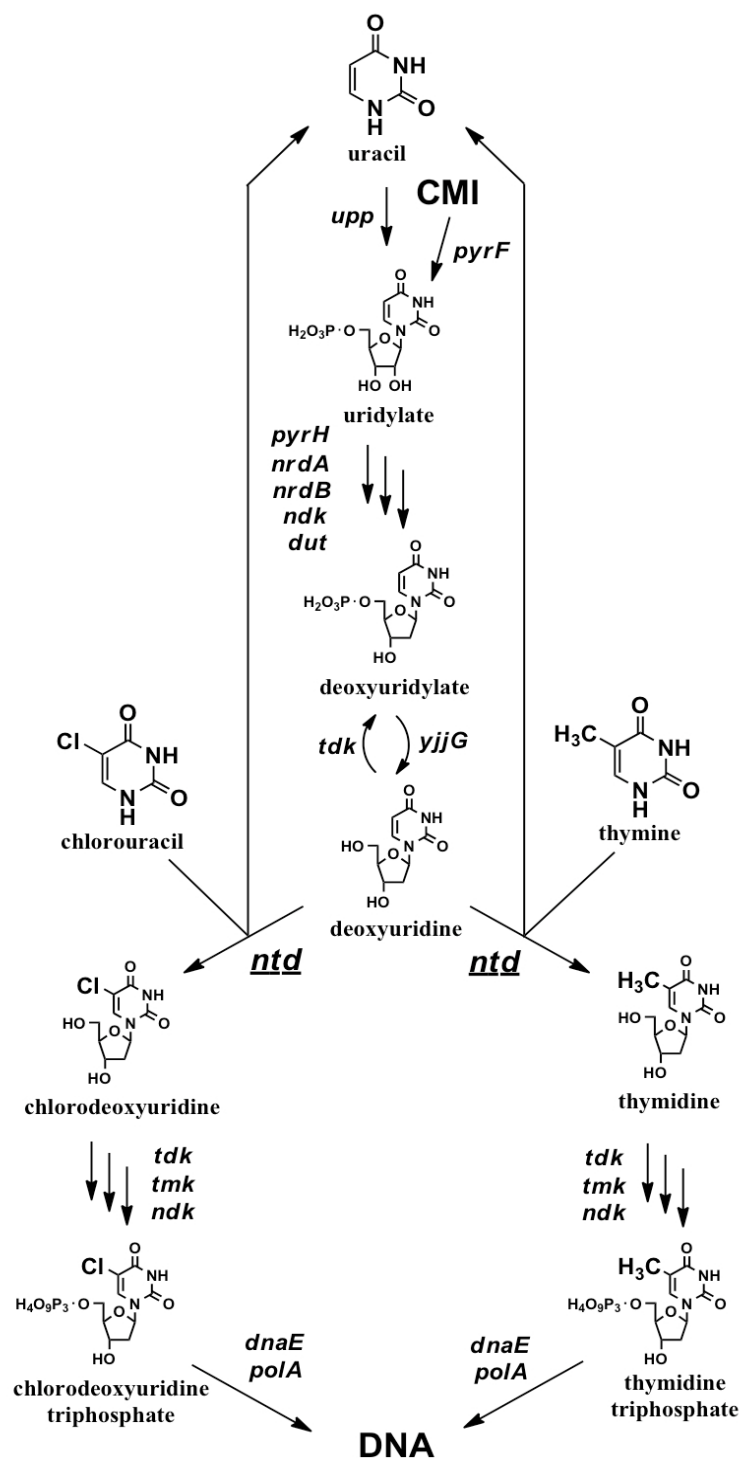


Figure S1b)

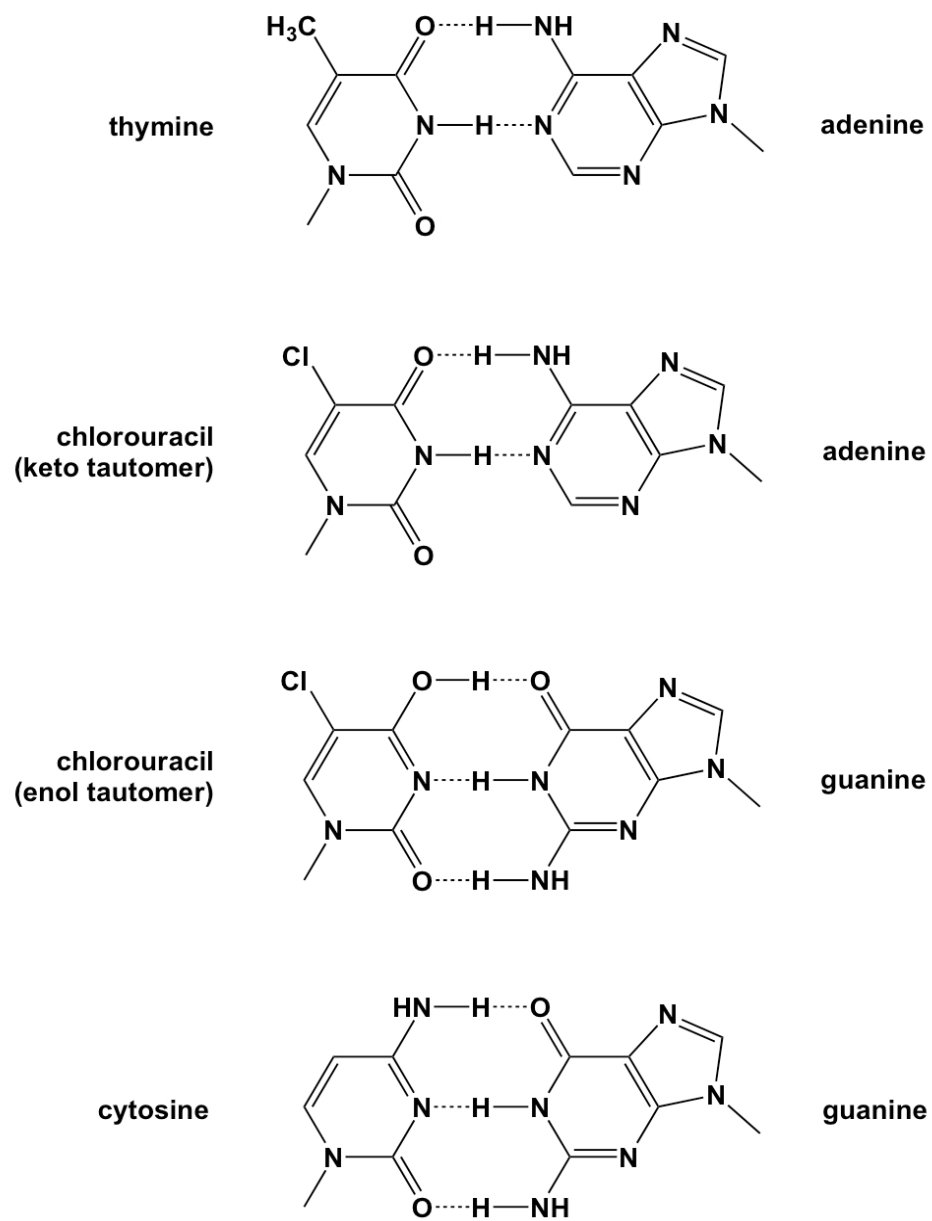


Figure S1c)

Figure S1: Parallel metabolic conversion of thymine (5-methyluracil) and 5-chlorouracil to DNA nucleotides and compared schemes of chlorouracil pairing with adenine and mispairing with guanine. **a)** Biosynthesis and salvage of the precursor deoxyuridylate (dUMP) to the DNA monomer thymidine triphosphate (dTTP) in wild-type *E. coli*. Enzymatic steps are indicated by the names of the corresponding genes. The key enzyme thymidylate synthase, encoded by *thyA*, converts dUMP into dTMP and concomitantly methylenetetrahydrofolate into dihydrofolate. Enzymes encoded by the *deo* operon recycle dU into uracil, to be further converted to the RNA precursor UMP, and into central metabolism intermediates (CMI), phosphoglyceraldehyde and acetaldehyde. **b)** Concurrent biosynthesis of the competing DNA monomers d χ TP and dTTP from dUMP in the THY1 strain. Expression of the *ntd* gene for nucleoside deoxyribosyltransferase from *Lactobacillus leichmannii* enables the reversible transfer of deoxyribosyl moieties from deoxyuridine to either thymine or chlorouracil. Underlined gene names in **a)** and **b)** indicate metabolic steps that were altered for constructing the strain THY1 from the wt reference MG1655 by chromosomal deletion or plasmid insertion. **c)** Compared schemes of chlorouracil pairing with adenine and mispairing with guanine.

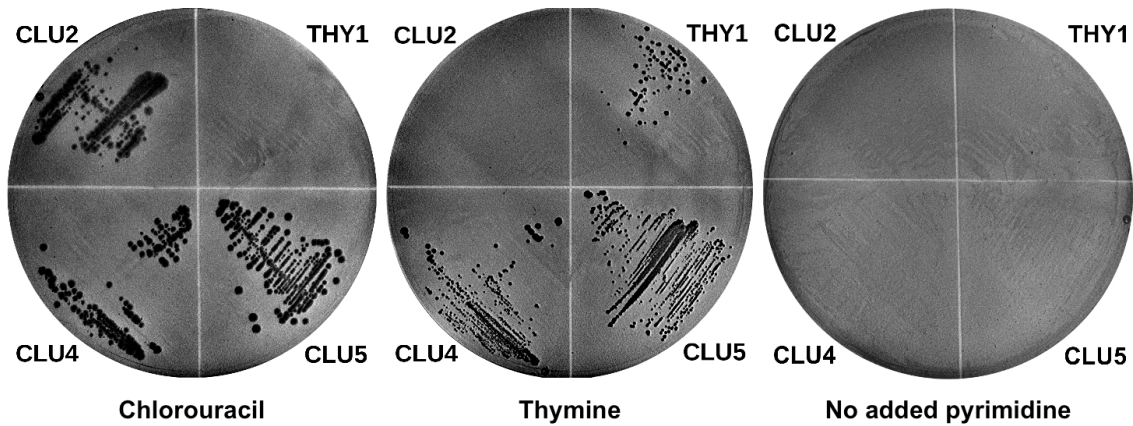


Figure S2: Growth phenotype of chlorouracil-adapted descendants of *E. coli*. Bacterial cells were plated on semi-solid MS glucose 0.2% medium containing thymine (3 μ M), chlorouracil (3 μ M) or no added pyrimidine. Petri dishes were photographed after a 48h-incubation at 37°C. The numerical images were converted to black and white and inverted using GIMP (GNU Image Manipulation Program). An interesting aspect of base translocation is that it affects not only the genotype but also the phenotype of DNA. Considering that a thymine site can be occupied partially by chlorouracil and that the *E. coli* genome features over 2 million such sites, it follows that a given configuration of partial chlorouracil replacement never occurs twice in the adapting cultures before they reach complete replacement.

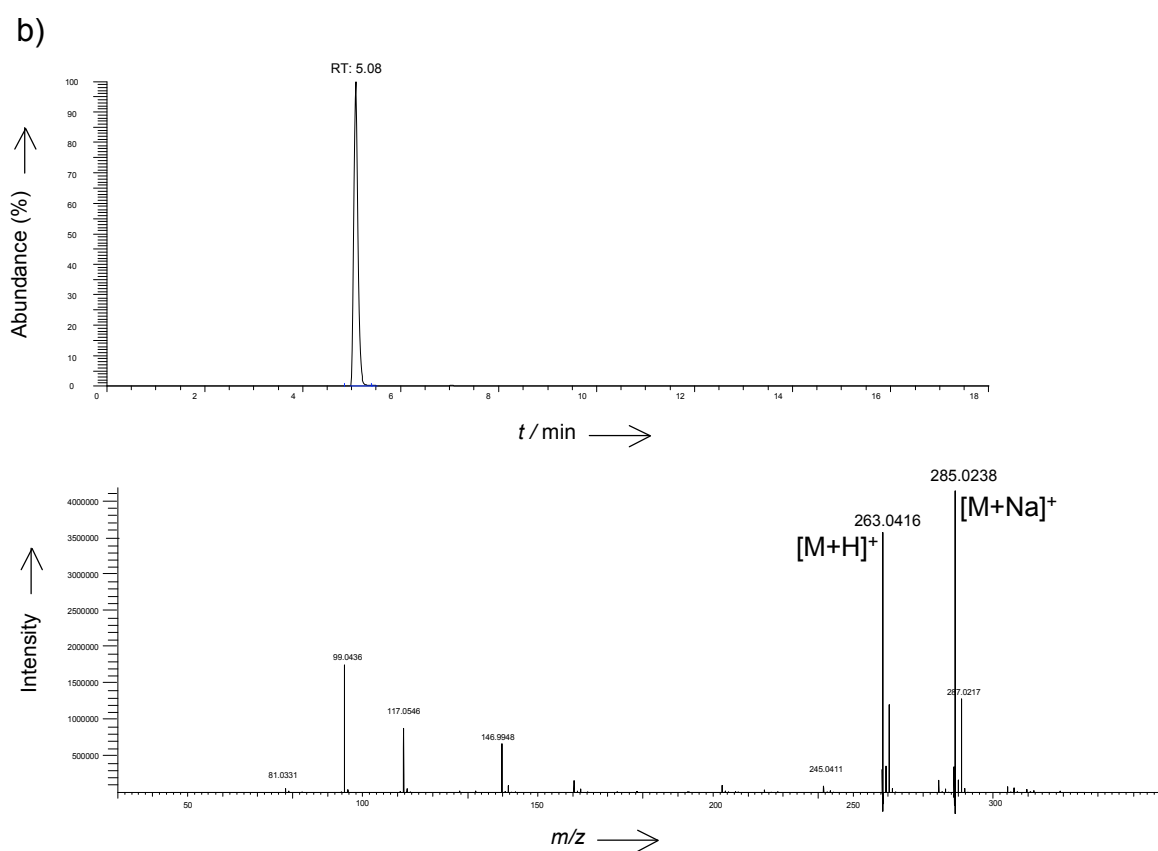
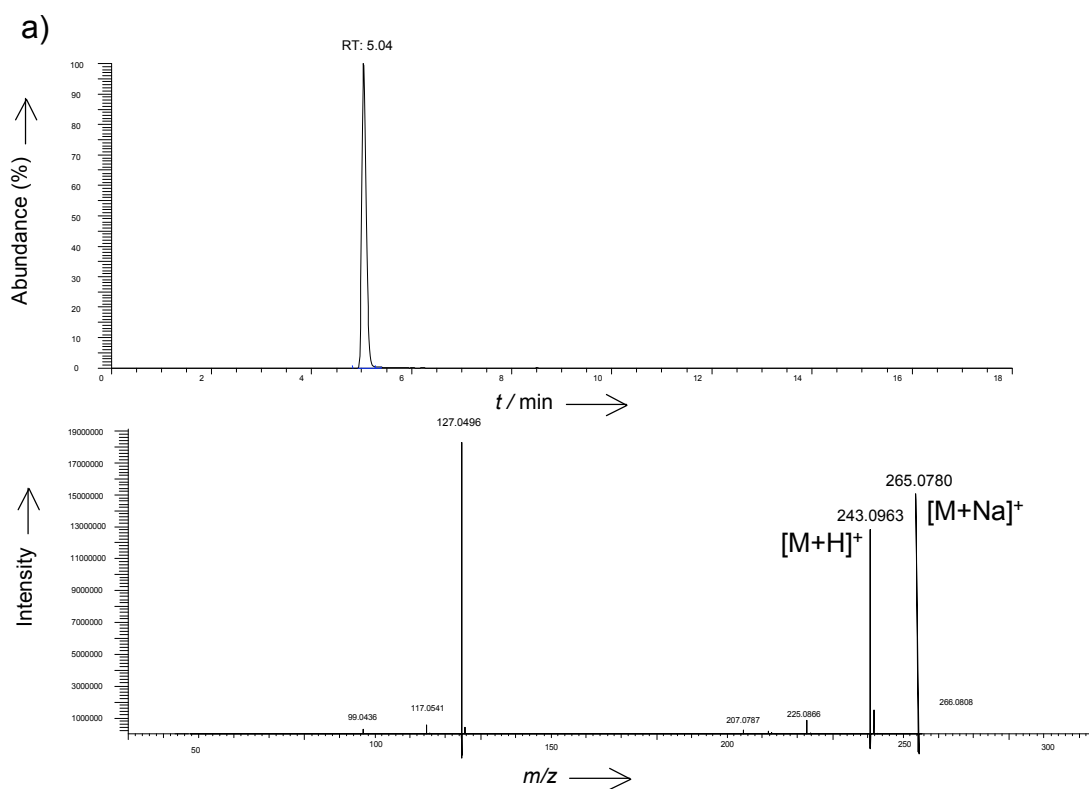


Figure S3: Chromatogram and mass spectrum of thymidine and of 5-chloro-2'-deoxyuridine. Samples were analyzed using a C-18 column (100 x 2 mm x 1.7 μ m, Acquity BeH, Waters) using a gradient (solvent A : ammonium acetate 10 mM, HCOOH 1%, solvent B : methanol) at a flow rate of 400 μ L/min (2 min solvent A, linear gradient to solvent B in 8 min, 2 min solvent B). Detection: Orbitrap mass spectrometer (HESI source, 4kV, 150 C). Because thymidine and 5-chloro-2'-deoxyuridine are not separated in the HPLC system, we used the [M+H]⁺ signal (respectively 243.0982 and 263.0435) to assign their presence in an unambiguous way. **a)** Thymidine chromatogram (upper part) and mass-spectrum (lower part); **b)** 5-Chloro-2'-deoxyuridine chromatogram (upper part) and mass-spectrum (lower part).

Concentration (μM)	dT	d χ
0	0	0
1	330418	92211
2	777456	183810
4	1825618	408897
6	2854728	713931
8	4045148	970511
10	5073400	1213425
50	27029636	6400250
100	47326319	13812584
250	89985424	31570123
500	138351200	56923661
1000	213957422	89602418

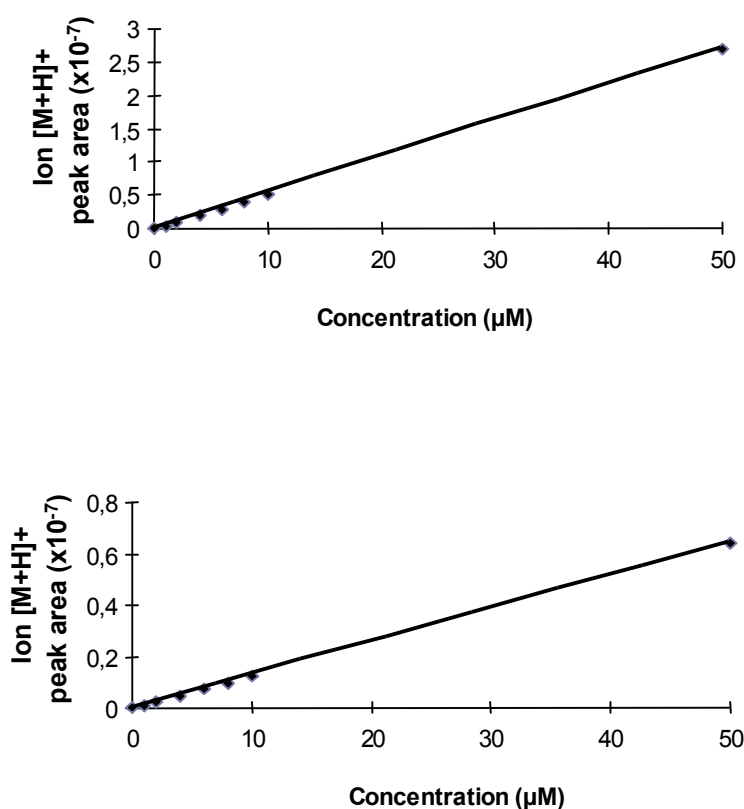


Figure S4: Calibrating curves obtained for the mixtures of thymidine and 5-chloro-2'-deoxyuridine (concentrations 0 to 1 000 μM). The table gives the peak area of the $[\text{M}+\text{H}]^+$ ion, figures show regression lines for thymidine (dT, upper part) and 5-chloro-2'-deoxyuridine (d χ , lower part).