Supplementary information

**Thiouridine residues in tRNAs are responsible for a synergistic effect of UVA and UVB light in photoinactivation of *Escherichia coli***

Sandra Probst-Rüda b, Kristopher McNeilla \*, Martin Ackermanna b \*

a Institute of Biogeochemistry and Pollutant Dynamics (IBP), Department of Environmental Systems Science, ETH Zurich, Zurich, Switzerland

b Department of Environmental Microbiology, Swiss Federal Institute of Aquatic Science and Technology (Eawag), Dübendorf, Switzerland

\* equal contribution; co-corresponding authors

For correspondence.

E-mail: martin.ackermann@env.ethz.ch; Tel. +4158 765 5122; Fax +4158 765 55 47.

E-mail: kris.mcneill@env.ethz.ch; Tel. +4144 632 4755; Fax +4144 632 1438.

SI Table 1: Strains used in this study 3

SI Table 2: List of all mutations identified in the 10 evolved strains compared to the corresponding ancestor. 4

SI Table 3: Primer list of all primers used for the construction of strains SP21-SP32 10

SI Figure 1: Relative light intensity spectra of the UVA lamp (a) and the UVB lamp (b). The light intensity was normalized to 1 at 365 nm (UVA lamp) and 311 nm (UVB lamp) respectively. 3

SI Figure 2: The shown survival rates represent the sensitivity towards UV irradiation after eight hours of irradiation. The 12 populations were irradiated the eighth consecutive day while ancestor1 was not irradiated previously. 9

SI Figure 3: Comparison of the survival ratio for strains with different alleles in *thiI*. The strains between SP01 and SP12 are evolved strains from the selection experiment. The strains between SP21 and SP32 are ancestor strains harboring a mutant version of *thiI.* Error bars represent the standard errors of the means. 10



SI Figure 1: Relative light intensity spectra of the UVA lamp (a) and the UVB lamp (b). The light intensity was normalized to 1 at 365 nm (UVA lamp) and 311 nm (UVB lamp) respectively.

SI Table 1: Strains used in this study

|  |  |  |  |
| --- | --- | --- | --- |
| Strain | Genotype | Parental strain | Reference |
| wt | E. *coli* MG1655 |  | (Blattner et al., 1997) |
| ancestor1 | Δ*lacZ*::*rfp* | E. *coli* MG1655 | This study |
| ancestor2 | Δ*lacA*::*gfp* | E. *coli* MG1655 | This study |
| SP40 | Δ*thiI*::*kan* | E. *coli* MG1655 | (Baba et al., 2006) |
|  |  |  |  |
| *Strains isolated from selection experiment (further mutations list in SI Table 2)* | | | |
| SP01 | *thiI* A41T  *thiI* C815A  *thiI* C815A  *thiI* C554T  *thiI* C578T  *thiI* G862A  *thiI* G862A  *thiI* G1024A  *thiI* G1024A  *thiI* GA 28-29 AG | Ancestor1 | This study |
| SP02 | Ancestor2 | This study |
| SP03 | Ancestor1 | This study |
| SP04 | Ancestor2 | This study |
| SP07 | Ancestor1 | This study |
| SP08 | Ancestor2 | This study |
| SP09 | Ancestor1 | This study |
| SP10 | Ancestor2 | This study |
| SP11 | Ancestor1 | This study |
| SP12 | Ancestor2 | This study |
|  |  |  |  |
| *Strains constructed by allelic replacement* | |  |  |
| SP21 | *thiI* A41T | E. *coli* MG1655 | This study |
| SP22 | *thiI* C815A | Ancestor1 | This study |
| SP23 | *thiI* C815A | E. *coli* MG1655 | This study |
| SP24 | *thiI* C554T | Ancestor1 | This study |
| SP27 | *thiI* C578T | E. *coli* MG1655 | This study |
| SP28 | *thiI* G862A | Ancestor1 | This study |
| SP29 | *thiI* G862A | E. *coli* MG1655 | This study |
| SP30 | *thiI* G1024A | Ancestor1 | This study |
| SP31 | *thiI* G1024A | E. *coli* MG1655 | This study |
| SP32 | *thiI* GA 28-29 AG | Ancestor1 | This study |

SI Table 2: List of all mutations identified in the 10 evolved strains compared to the corresponding ancestor.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Strain | Position | Mutation | Annotation | Gene |
| SP01 | 119,896 | Δ3 bp | coding (616‑618/855 nt) | *ampE* → |
| SP01 | 440,813 | A→T | K14I (AAA→ATA) | *thiI* → |
| SP01 | 831,883 | Δ3 bp | coding (289‑291/483 nt) | *ybiA* ← |
| SP01 | 1,396,660 | Δ3 bp | intergenic (‑14/+136) | *uspE* ← / ← *fnr* |
| SP01 | 1,484,505 | Δ4 bp | coding (3421‑3424/3903 nt) | *hrpA* → |
| SP01 | 1,880,643 | Δ3 bp | coding (708‑710/1086 nt) | *dmlA* → |
| SP01 | 2,423,266 | Δ3 bp | coding (671‑673/687 nt) | *hisQ* ← |
| SP01 | 2,426,540 | Δ3 bp | coding (107‑109/570 nt) | *ubiX* ← |
| SP01 | 3,133,999 | Δ6 bp | coding (390‑395/1500 nt) | *pitB* ← |
| SP01 | 3,186,567 | Δ3 bp | pseudogene (1319‑1321/3805 nt) | *yqiG* → |
| SP01 | 3,244,484 | Δ12 bp | coding (1359‑1370/1419 nt) | *exuT* → |
| SP01 | 3,371,541 | G→A | Q20\* (CAA→TAA) | *nanA* ← |
| SP01 | 3,688,311 | Δ3 bp | coding (2318‑2320/2340 nt) | *bcsB* ← |
| SP01 | 3,956,672 | Δ4 bp | coding (680‑683/1476 nt) | *ilvC* → |
| SP01 | 4,160,722 | Δ4 bp | coding (569‑572/1101 nt) | *trmA* ← |
|  |  |  |  |  |
| SP02 | 441,587 | C→A | A272E (GCA→GAA) | *thiI* → |
| SP02 | 965,156 | G→A | L538L (CTG→CTA) | *ycaI* → |
| SP02 | 1,379,600 | C→T | pseudogene (327/1082 nt) | *ycjV* → |
| SP02 | 1,666,422 | T→C | E56G (GAA→GGA) | *mlc* ← |
| SP02 | 2,990,422 | G→A | G103R (GGG→AGG) | *ygeH* → |
|  |  |  |  |  |
| SP03 | 441,326 | C→T | S185F (TCC→TTC) | *thiI* → |
| SP03 | 1,195,443 | C→T | H366H (CAC→CAT) | *icd* → |
| SP03 | 1,195,455 | C→T | T370T (ACC→ACT) | *icd* → |
| SP03 | 1,195,468 | T→C | L375L (TTA→CTG) | *icd* → |
| SP03 | 1,195,470 | A→G | L375L (TTA→CTG) | *icd* → |
| SP03 | 1,195,500 | C→T | N385N (AAC→AAT) | *icd* → |
| SP03 | 1,195,503 | G→C | A386A (GCG→GCC) | *icd* → |
| SP03 | 1,195,506 | A→G | K387K (AAA→AAG) | *icd* → |
| SP03 | 1,299,502 | C→G | T99T (ACC→ACG) | *oppA* → |
| SP03 | 1,379,943 | T→G | intergenic (‑17/‑28) | *ycjV* → / → *ompG* |
| SP03 | 1,603,237 | G→A | E55K (GAG→AAG) | *lsrB* → |
| SP03 | 2,783,653 | T→A | intergenic (‑620/+131) | *ypjC* ← / ← *ileY* |
| SP03 | 2,783,664 | C→T | intergenic (‑631/+120) | *ypjC* ← / ← *ileY* |
| SP03 | 2,875,564 | G→T | G321V (GGA→GTA) | *iap* → |
|  |  |  |  |  |
| SP04 | 441,326 | C→T | S185F (TCC→TTC) | *thiI* → |
| SP04 | 583,842 | T→G | intergenic (+189/+61) | *appY* → / ← *ompT* |
| SP04 | 583,851 | C→T | intergenic (+198/+52) | *appY* → / ← *ompT* |
| SP04 | 1,190,636 | C→T | E192K (GAG→AAG) | *purB* ← |
| SP04 | 1,195,443 | C→T | H366H (CAC→CAT) | *icd* → |
| SP04 | 1,195,455 | C→T | T370T (ACC→ACT) | *icd* → |
| SP04 | 1,195,468 | T→C | L375L (TTA→CTG) | *icd* → |
| SP04 | 1,195,470 | A→G | L375L (TTA→CTG) | *icd* → |
| SP04 | 1,898,419 | G→A | I397I (ATC→ATT) | *yoaE* ← |
| SP04 | 3,491,924 | C→T | intergenic (+153/‑109) | *tsgA* → / → *nirB* |
|  |  |  |  |  |
| SP07 | 441,350 | C→T | S193F (TCC→TTC) | *thiI* → |
| SP07 | 1,195,443 | C→T | H366H (CAC→CAT) | *icd* → |
| SP07 | 1,195,455 | C→T | T370T (ACC→ACT) | *icd* → |
| SP07 | 1,195,468 | T→C | L375L (TTA→CTG) | *icd* → |
| SP07 | 1,195,470 | A→G | L375L (TTA→CTG) | *icd* → |
| SP07 | 1,195,500 | C→T | N385N (AAC→AAT) | *icd* → |
| SP07 | 1,195,503 | G→C | A386A (GCG→GCC) | *icd* → |
| SP07 | 1,195,506 | A→G | K387K (AAA→AAG) | *icd* → |
| SP07 | 1,195,515 | C→T | T390T (ACC→ACT) | *icd* → |
| SP07 | 1,351,487 | C→T | M56M (GTG→ATG) | *sapD* ← |
| SP07 | 1,410,536 | C→T | D242N (GAT→AAT) | *intR* ← |
|  |  |  |  |  |
| SP08 | 181,690 | G→A | P269P (CCG→CCA) | *degP* → |
| SP08 | 366,933 | Δ39 bp | coding (674‑712/834 nt) | *mhpR* ← |
| SP08 | 441,634 | G→A | E288K (GAA→AAA) | *thiI* → |
| SP08 | 754,164 | C→A | intergenic (‑473/‑236) | *gltA* ← / → *sdhC* |
| SP08 | 877,433 | T→C | intergenic (‑175/‑38) | *rimO* ← / → *bssR* |
| SP08 | 1,570,856 | C→T | D791N (GAT→AAT) | *pqqL* ← |
| SP08 | 1,886,015 | A→G | M1M (TTG→CTG) | *rnd* ← |
| SP08 | 2,333,620 | C→T | G349G (GGG→GGA) | *yfaA* ← |
| SP08 | 3,602,891 | A→G | E159G (GAA→GGA) | *rsmD* → |
| SP08 | 4,045,092 | Δ33 bp | coding (104‑136/2787 nt) | *polA* → |
| SP08 | 4,136,491 | Δ3 bp | coding (566‑568/1104 nt) | *gldA* ← |
| SP08 | 4,185,300 | A→G | D643G (GAC→GGC) | *rpoC* → |
|  |  |  |  |  |
| SP09 | 441,634 | G→A | E288K (GAA→AAA) | *thiI* → |
| SP09 | 1,791,355 | G→C | A76A (GCC→GCG) | *btuD* ← |
| SP09 | 1,802,994 | G→A | pseudogene (24/1900 nt) | *arpB* → |
| SP09 | 3,436,067 | C→T | R8C (CGC→TGC) | *mscL* → |
| SP09 | 3,906,461 | C→A | intergenic (‑72/+111) | *pstB* ← / ← *pstA* |
|  |  |  |  |  |
| SP10 | 261,229 | T→C | L168S (TTA→TCA) | *proA* → |
| SP10 | 438,083 | A→T | L440Q (CTG→CAG) | *dxs* ← |
| SP10 | 441,796 | G→A | E342K (GAA→AAA) | *thiI* → |
| SP10 | 1,195,443 | C→T | H366H (CAC→CAT) | *icd* → |
| SP10 | 1,195,455 | C→T | T370T (ACC→ACT) | *icd* → |
| SP10 | 1,195,468 | T→C | L375L (TTA→CTG) | *icd* → |
| SP10 | 1,195,470 | A→G | L375L (TTA→CTG) | *icd* → |
| SP10 | 1,195,500 | C→T | N385N (AAC→AAT) | *icd* → |
| SP10 | 1,195,503 | G→C | A386A (GCG→GCC) | *icd* → |
| SP10 | 1,195,506 | A→G | K387K (AAA→AAG) | *icd* → |
| SP10 | 1,195,515 | C→T | T390T (ACC→ACT) | *icd* → |
| SP10 | 1,195,527 | G→A | E394E (GAG→GAA) | *icd* → |
| SP10 | 1,412,828 | C→T | K861K (AAG→AAA) | *recE* ← |
| SP10 | 1,500,251 | +T | intergenic (+72/‑230) | *tehB* → / → *ydcL* |
| SP10 | 1,682,194 | T→A | F430Y (TTT→TAT) | *rstB* → |
| SP10 | 1,859,197 | C→T | E54K (GAG→AAG) | *ydjL* ← |
| SP10 | 1,870,478 | C→T | R138R (CGC→CGT) | *yeaJ* → |
| SP10 | 1,871,630 | T→A | H11Q (CAT→CAA) | *yeaK* → |
|  |  |  |  |  |
| SP11 | 441,796 | G→A | E342K (GAA→AAA) | *thiI* → |
|  |  |  |  |  |
| SP12 | 440,800 | 2 bp→AG | coding (28‑29/1449 nt) | *thiI* → |
| SP12 | 2,147,053 | G→A | intergenic (+3/+10) | *yegD* → / ← *yegI* |
| SP12 | 2,193,155 | C→T | F278F (TTC→TTT) | *metG* → |
| SP12 | 2,953,473 | C→T | E185K (GAA→AAA) | *recB* ← |
| SP12 | 3,888,369 | C→T | A37A (GCC→GCT) | *tnaB* → |

SI Figure 2: The shown survival rates represent the sensitivity towards UV irradiation after eight hours of irradiation. The 12 populations were irradiated the eighth consecutive day while ancestor1 was not irradiated previously.



SI Figure 3: Comparison of the survival ratio for strains with different alleles in *thiI*. The strains between SP01 and SP12 are evolved strains from the selection experiment. The strains between SP21 and SP32 are ancestor strains harboring a mutant version of *thiI.* Error bars represent the standard errors of the means.

SI Table 3: Primer list of all primers used for the construction of strains SP21-SP32

|  |  |  |  |
| --- | --- | --- | --- |
| **Name** | **aSequence (5’🡪3’)** | **Construction of strain** | **Used for** |
| thiI-mut1-f | gaagatctAGTTGCTGCGGAAAGTCCAT | SP21 | Mutation construction |
| thiI-mut1-r | gaagatctCAATACCTTCGTAGCGGCCT | SP21 | Mutation construction |
| thiI-seq1 | CGTTCAGCGCCTCTTC | SP21 | Fragment sequencing |
| thiI-NLS1-f | ggCGGAAATCACCATCAT | SP21 | Genotyping (TSP) |
| thiI-NLS1-r | ggGGAATACGGGTCAGA | SP21 | Genotyping (TSP) |
| thiI-LS1-f | CTCTTCCAGCGGCAGGTCG | SP21 | Genotyping (TSP) |
| thiI-LS1-r | GATGGACAGTCACATCCGGATTG | SP21 | Genotyping (TSP) |
|  |  |  |  |
| thiI-mut2-f | gaagatctGATGTGGAACGTTACGTCGG | SP22, SP23 | Mutation construction |
| thiI-mut2-r | gaagatctTCTGGTCGAGATCGCCAAAT | SP22, SP23 | Mutation construction |
| thiI-seq2 | AATCAGCATATTGAATCCGC | SP22, SP23 | Fragment sequencing |
| thiI-NLS2-f | ggTCAGCCACTTTAGATT | SP22, SP23 | Genotyping (TSP) |
| thiI-NLS2-r | gcGCATTATCTGTGGAAC | SP22, SP23 | Genotyping (TSP) |
| thiI-LS2-f | TTTCCACTTCCACCACTTCCTGC | SP22, SP23 | Genotyping (TSP) |
| thiI-LS2-r | GGCGGCGGTTTAAATCAGCATAT | SP22, SP23 | Genotyping (TSP) |
|  |  |  |  |
| thiI-mut4-f | gaagatctAAAGCCAATCTGTGCGCTTG | SP24 | Mutation construction |
| thiI-mut4-r | gaagatctCTTTCACCGTCGGGCTTTTG | SP24 | Mutation construction |
| thiI-seq4 | TCCACCATATTCTCGAAGTC | SP24 | Fragment sequencing |
| thiI-NLS4-f | cgTCGAAACCACCGA | SP24 | Genotyping (TSP) |
| thiI-NLS4-r | ccCGGTTTAAATCAGCAT | SP24 | Genotyping (TSP) |
| thiI-LS4-f | CAGGATCAGCGTGTCGGAGA | SP24 | Genotyping (TSP) |
| thiI-LS4-r | CCTTACCGGGAACATTCGTAACG | SP24 | Genotyping (TSP) |
|  |  |  |  |
| thiI-mut7-f | gaagatctAAAGCCAATCTGTGCGCTTG | SP27 | Mutation construction |
| thiI-mut7-r | gaagatctCTTTCACCGTCGGGCTTTTG | SP27 | Mutation construction |
| thiI-seq7 | TATCAATCAGGCGCAGGT | SP27 | Fragment sequencing |
| thiI-NLS7-f | ggGCATCAACATATAACTGA | SP27 | Genotyping (TSP) |
| thiI-NLS7-r | ccCTCCTGCTGATTAAAG | SP27 | Genotyping (TSP) |
| thiI-LS7-f | GAAAGCGTTGGTTCAGTATCGCG | SP27 | Genotyping (TSP) |
| thiI-LS7-r | TCGATTTAACTGCTTTCACCGTCG | SP27 | Genotyping (TSP) |
|  |  |  |  |
| thiI-mut8-f | gaagatctGATGTGGAACGTTACGTCGG | SP28, SP29 | Mutation construction |
| thiI-mut8-r | gaagatctTCTGGTCGAGATCGCCAAAT | SP28, SP29 | Mutation construction |
| thiI-seq8 | AATCAGCATATTGAATCCGC | SP28, SP29 | Fragment sequencing |
| thiI-NLS8-f | cgTGGTCACCGGCA | SP28, SP29 | Genotyping (TSP) |
| thiI-NLS8-r | cgCACACCACAATATTCC | SP28, SP29 | Genotyping (TSP) |
| thiI-LS8-f | AAAGGCCGCTACGAAGGTATTGG | SP28, SP29 | Genotyping (TSP) |
| thiI-LS8-r | AGAACGGCAGAGAAACCACATCA | SP28, SP29 | Genotyping (TSP) |
|  |  |  |  |
| thiI-mut10-f | gaagatctGCTACGAAGGTATTGGCGGT | SP30, SP31 | Mutation construction |
| thiI-mut10-r | gaagatctGCGTGAAAAAGCCCATGAAGT | SP30, SP31 | Mutation construction |
| thiI-seq10 | TCCAGTTATATGTTGATGCG | SP30, SP31 | Fragment sequencing |
| thiI-NLS10-f | ccGATCACACCACAATATTT | SP30, SP31 | Genotyping (TSP) |
| thiI-NLS10-r | gcGCGCCTGATTGATAA | SP30, SP31 | Genotyping (TSP) |
| thiI-LS10-f | GGTGATGGCGGCAGGGATTTATA | SP30, SP31 | Genotyping (TSP) |
| thiI-LS10-r | TATATGTTGATGCGTCGCGGCTG | SP30, SP31 | Genotyping (TSP) |
|  |  |  |  |
| thiI-mut12-f | gaagatctAGTTGCTGCGGAAAGTCCAT | SP32 | Mutation construction |
| thiI-mut12-r | gaagatctCAATACCTTCGTAGCGGCCT | SP32 | Mutation construction |
| thiI-seq12 | TCGGCATAATGAATGTCTGA | SP32 | Fragment sequencing |
| thiI-NLS12-f | TTTATCATTAAATTGTTCC | SP32 | Genotyping (TSP) |
| thiI-NLS12-r | TCTCATCATAGTGCTT | SP32 | Genotyping (TSP) |
| thiI-LS12-f | CAGACAGCAGAATTTGTACGCGC | SP32 | Genotyping (TSP) |
| thiI-LS12-r | TCGTAGCGGCCTTTAATCAGCAG | SP32 | Genotyping (TSP) |
|  |  |  |  |
| pKOV-unstuff-f | AGGGCAGGGTCGTTAAATAGC | All strains | Sequencing from vector |
| pKOV-unstuff-r | TTAATGCGCCGCTACAGGGCG | All strains | Sequencing from vector |

aCapital letters=pairs with target sequence, small letters=does not pair (*i.e.*, tail). BglII restriction sites (AGATCT) underlined