

# Identification of a VapBC toxin antitoxin system in a thermophilic bacterium *Thermus thermophilus* HB27

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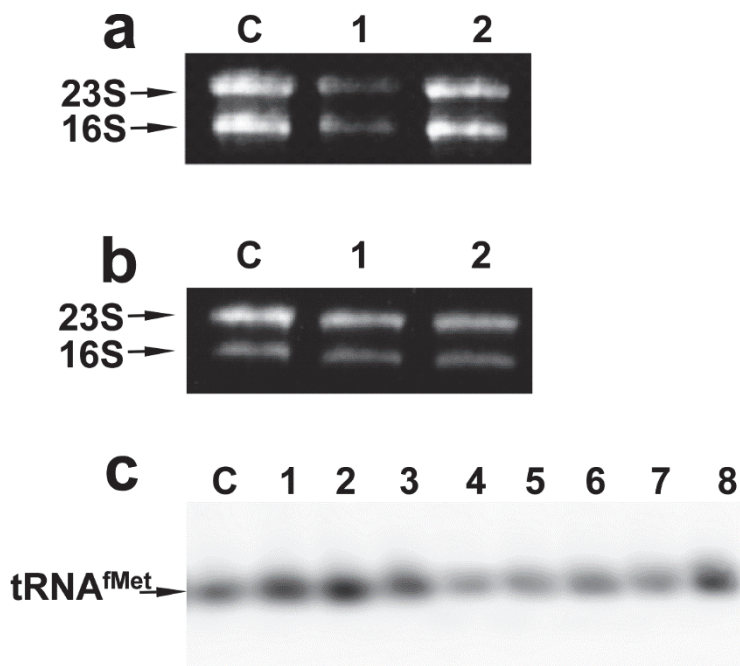
**Table S1** Primers used in this study

Primer	Sequence (5'-3') <sup>a</sup>
<i>for cloning into pZE21MCS</i>	
TTC0113F-Kpn	AAAGGTACCATGGGAAGGCGCTACCTCCTAGAC
TTC0113R-Sal	AAAGTCGACTCACCACAGGGTCTCCACGC
TTC0114F-Kpn	AAAGGTACCATGAAGGCCTTCACCGTGAC
TTC0125F-Kpn	AAAGGTACCATGGTGTGCTGGACGCTTCCGC
TTC0125R-Sal	AAAGTCGACTCAGGAGGGCTTCCACGCCA
TTC0126F-Kpn	AAAGGTACCATGGCCCTCACCATCCGCAAC
TTC1207F-Kpn	AAAGGTACCATGAGGGTTCGTCCTGGACACC
TTC1207R-Sal	AAAGTCGACTCAGCTCACCTCCCGCAAAAAG
TTC1208F-Kpn	AAAGGTACCATGCGGGCGCATTGCCCTGC
TTC1395F-Kpn	AAAGGTACCGTGAGCCCCCGCCTCATCC
TTC1395R-Sal	AAAGTCGACTCAACGGGATCCAAGGCGCTTTTG
TTC1394F-Kpn	AAAGGTACCAGATGAGGCGGGCGTTACCG
TTC1549F-Kpn	AAAGGTACCATGGCCAAGGGGGGGCAC
TTC1549R-Sal	AAAGTCGAC CTAGAGGTTATGGA ACTC
TTC1548F-Kpn	AAAGGTACCATGCTCAAGTACACCGCCCTCC
TTC1705F-Kpn	AAAGGTACCATGGCGAGGCGGCTTAGGC
TTC1705R-Sal	AAAGTCGACTCACTCCAGCCATTCCCTCCTCCTC
TTC1704F-Kpn	AAAGGTACCATGGACGGGATGGGCACCCTG
TTC1805F-Kpn	AAAGGTACCATGATGCATCGCAAGCGCGTC
TTC1805R-Sal	AAAGTCGACCTAGCGTCCAGCACCACCAG
TTC1804F-Kpn	AAAGGTACCATGAGCTGGGTGGTGTGCTGGACGC
<i>for cloning into pET28a</i>	
TTC0126F-Nde	AAACATATGGCCCTCACCATCCGCAAC
TTC0126R-Xho	AAACTCGAGTCAGTACCCCTCCTTGCCGTAC
TTC0125R-Xho	AAACTCGAGTCAGGAGGGCTTCCACGCCA
tetRF-Nde	CCGGCATATGGCACGGCTGAACAGAGA
tetRR-Xho	CCGGCTCGAGTCAGCAAAAGGGGATGATAAG
TTP0042F-Nde	GAGAGCATATGACCGAGAACGCCGAAAATTC
TTP0042R-Xho	AATTCTCGAGTTAGGTCTGGGCCCGCGCG
<i>for Northern blotting</i>	
tRNAMet	biotin-CCATCATTATGAGTGATGTG
<i>for site-directed mutagenesis</i>	
D4AF	GCCATATGGTGTGCTGGCCGCTTCCGC
D4AR	GCGGAAGCGGCCAGCACCATATGGC
E23AF	GAGGAGCTTTTGGCGGAACTCCGGCG
E23AR	CGCCGGAGTTCCGCCAAAAGCTCCTC
E40AF	CCACCCTGGCCGAGCGGGGATCGTC
E40AR	GACGATCCCCGCTGCGGCCAGGGTGG
F71AF	CCGAGATCGTGCCCGCTACCGAAAGGCATG
F71AR	CATGCCTTTCGGTAGCGGGCACGATCTCGG
T72AF	GATCGTGCCCTTTGCCGAAAGGCATG
T72AR	CATGCCTTTCGGCAAAGGGCACGATC
A76DF	CCGAAAGGCATGACCGGGAGGCCATC

A76DR	GATGGCCTCCCGG <b>TC</b> ATGCCTTT <b>CGG</b>
G94DF	GGCACCCCGCCG <b>AT</b> CTCAACTTCGGG
G94DR	CCCGAAGTTGAG <b>AT</b> CGGC <b>GGGGT</b> GCC
G98DF	GGGCTCAACTTCGACG <b>ACT</b> GCCTGAGC
G98DR	GCTCAGGCAGTC <b>GTC</b> GAA <b>GT</b> TGAGCCC
D99AF	CTCAACTTCGGGGCCTGCCTGAGCTAC
D99AR	GTAGCTCAGGCAGG <b>CCCC</b> GAA <b>GT</b> TGAG
S102DF	GGGACTGCCTGG <b>ATT</b> ACGCCCTGGCC
S102DR	GGCCAGGGCGTA <b>ATCC</b> AGGCAGTCCC
L105DF	CTGAGCTACGCC <b>GAC</b> GCCCGGGTGGAG
L105DR	CTCCACCCGGG <b>CGTC</b> GGCGTAGCTCAG
V108DF	CCCTGGCCCGGG <b>AC</b> GAGGGGGAAACC
V108DR	GGT <b>CCCCCT</b> CG <b>TCCC</b> GGGCCAGGG
D119AF	CTACAAGGGCCAGGCCTTTGACCGGACG
D119AR	CGTCCGGTCAAAGGCCTGGCCCTTG <b>TAG</b>
D124AF	CTTTGACCGGACGGCCCTGGCGTGGAAG
D124AR	CTTCCACGCCAGGGCCGTCCGGTCAAAG

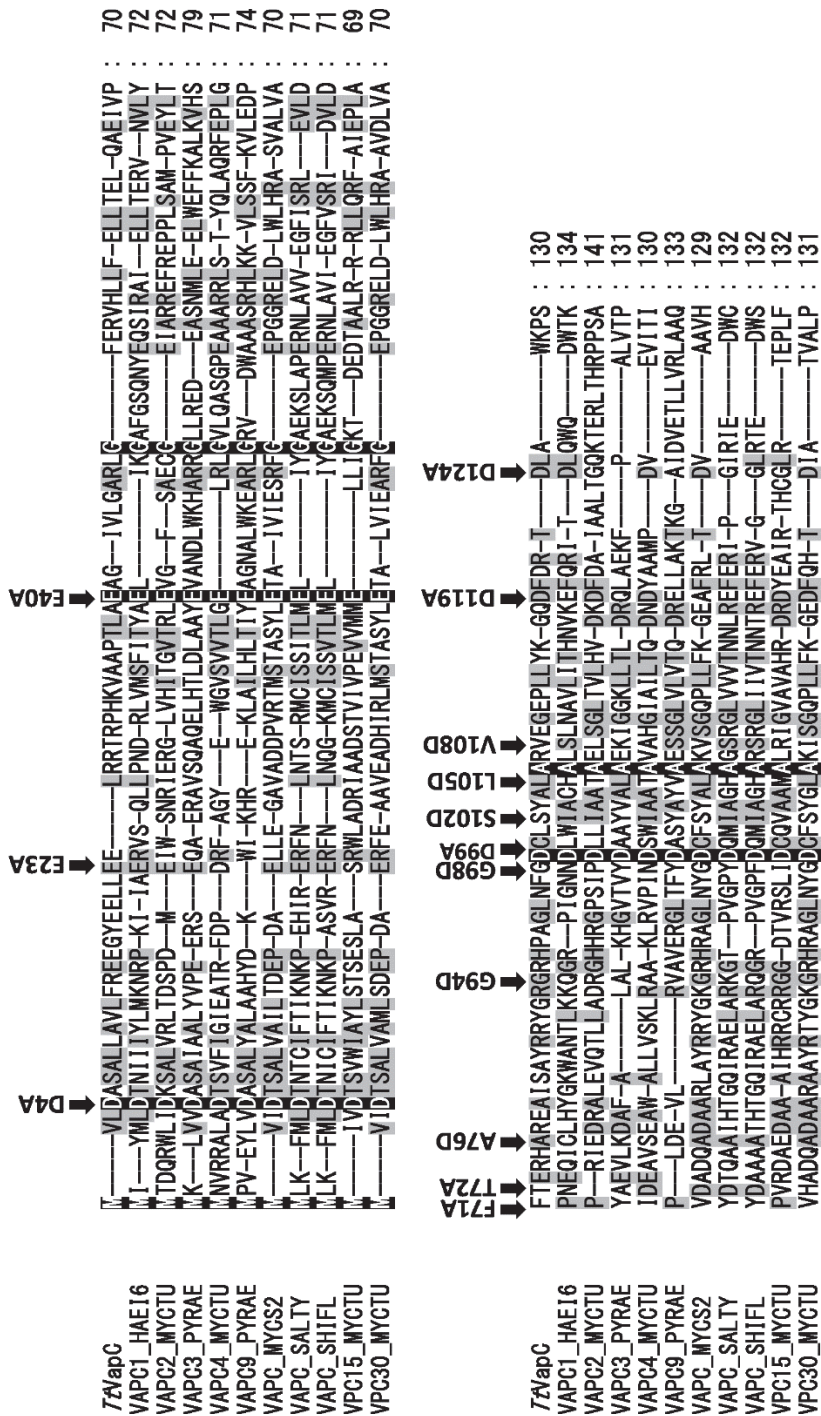
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<sup>a</sup>The restriction sites introduced are underlined, and the mutations are shown in italics.  
The initiation and termination codons are shown in bold.



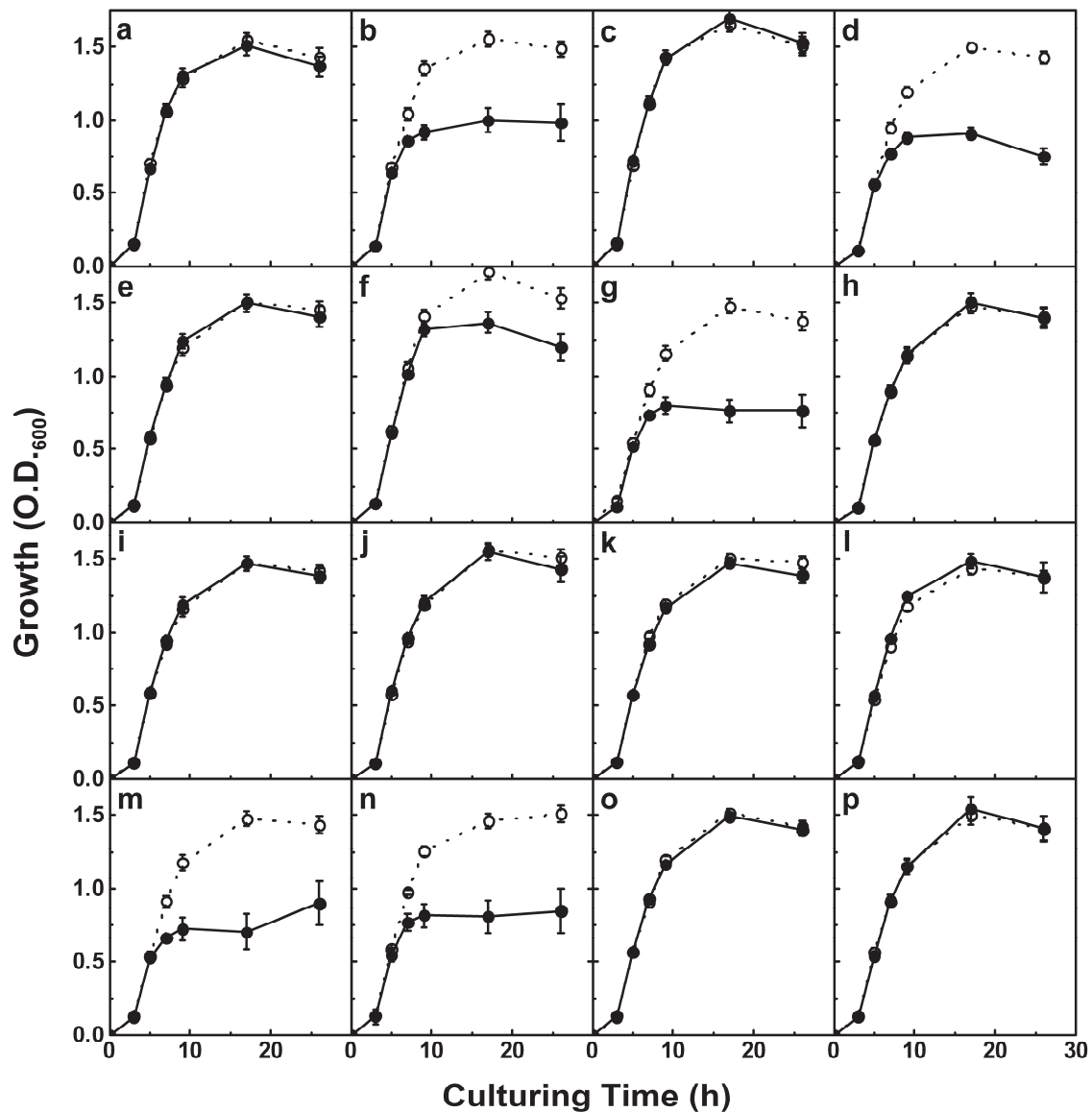
**Fig. S1** RNA degradation assay with intact ribosomes from *E. coli* (a), *T. thermophilus* (b), and with initiator tRNA (c)

(a, b) Intact ribosomes were incubated with *TtVapC* (lane 1) or the *TtVapBC* complex (lane 2) at 50 °C for 60 min. Then, RNA was prepared and loaded onto a denaturing agarose gel. Lane C indicates a control experiment in which ribosomes were incubated in the reaction buffer for 60 min. (c) Total RNA from *E. coli* was incubated with *TtVapC* for 0 (lane 1), 15 (lane 2), 30 (lane 3), or 60 min. (lane 4), or with the *TtVapBC* complex for 0 (lane 5), 15 (lane 6), 30 (lane 7), or 60 min. (lane 8), and resolved by denaturing PAGE. The initiator tRNA was detected by Northern blotting. Lane C indicates a control experiment without incubation with *TtVapC*. The positions of 23S, 16S rRNA, and  $tRNA^{fMet}$  are indicated by arrows.



**Fig. S2** Amino acid sequence alignment of *TtVapC* and the “reviewed” VapCs in the Uniprot database

The alignment was generated by the T-Coffee program. Conserved residues are shaded, of which those conserved among all of the sequences are in black. Amino acid substitutions introduced in *TtVapC* are indicated above the alignment.



**Fig. S3** Growth inhibition assay of *TtVapC* mutants

*E. coli* DH5 $\alpha$ Z1 harboring pZE21MCS (**a**), or the plasmid containing the wild type *Tt vapC* (**b**), D4A (**c**), E23A (**d**), E40A (**e**), F71A (**f**), T72A (**g**), A76D (**h**), G94D (**i**), G98D (**j**), D99A (**k**), S102D (**l**), L105D (**m**), V108D (**n**), D119A (**o**), or D124A (**p**) were cultured in LB medium containing kanamycin at 43 °C, and cell growth was monitored by measuring O.D.<sub>600</sub>. At O.D.<sub>600</sub>  $\approx$  0.2, Dox was added to induce gene expression. Open circles with dotted lines indicate the culture without induction, and filled circles with solid lines indicate the culture with induction. Culturing was conducted three times, and the mean values  $\pm$  standard deviations are shown.