TABLE S1 Oligonucleotides used in this study.

Oligonucleotide	Sequence (5'→3')[a]	Used for
P01	fwd, atacatatggtaacgacgacgagggaccgc;	construction of pET-ttPfmR
P02	rev, tatggatccttattaggcacttccacgttccaacccccctc	
P03; P04	fwd, aagcttgagtcttcgccgctcccctc; rev, atattctagacggtccctcgtcgtcgttac	construction of plasmid for *pfmR* gene
P05; P06	fwd, atatctgcagggttggaacgtggaagtgcc; rev, gaattctctccgggtcggtcttggcg	disruptant
P07	gtttcacgaggcgctgaaggacctcaa: positions 14,329–14,303 on pTT27	confirmation of replacement of the *pfmR*
P08	taggccgagcttccccttcagagggct: positions 12,617–12,591 on pTT27	gene by the kanamycin-resistance marker
P09	ggtacccgttgacggcggatatggtaca: positions 1–28 of the *HTK* gene	(*HTK*) gene on the genome of the Δ*pfmR*
P10	ctttaccgaaaatcgcttgatgagtgc: positions 98–72 of the *HTK* gene	strain
P11	cggatatatagtggatgtgtcaaaacgc: positions 998–1025 of the *HTK* gene	
P12	tgcagcgtaaccaacatgattaacaattat: positions 1080–1051 of the *HTK* gene	
P13	tggggctttccaaggccgccctcta: positions 101–125 of *pfmR* gene	
P14	atcgggccggaaccagcggatcat: positions 498–475 of *pfmR* gene	
P15	fwd, gatccggcccttgacggggccccggggagggagtagggtgggcttaccgaccggttggtcgtctggag;	construction of upstream region of the
P16	rev, aattctccagacgaccaaccggtcggtaagcccaccctactcccctccccggggccccgtcaagggccg	*TTHA0750* gene
P17	fwd, gatccgtcgcttgaccccgcccccgccttccggctaagctactaccgaccgttcggtaaggaggag;	construction of upstream region of the
P18	rev, aattctcctccttaccgaacggtcggtagtagcttagccggaaggcgggggcggggtcaagcgacg	*TTHA0987* gene
P19	fwd, gatcctggtcttgacgagaaagagaaaacggggtaaggtgaacctaccgaccggtcggtgggctatgg	construction of upstream region of the
P20	rev, aattccatagcccaccgaccggtcggtaggttcaccttaccccgttttctctttctcgtcaagaccag	*TTHB023* gene
P21	fwd, tgcatgcctgcaggtcgact	preparation of template DNA for the
P22	rev, gatcggtgcgggcctcttcg	transcription assay
P23	$_{p}$atcaccaccggatgg	cDNA synthesis of *TTHA0750* gene
P24	$_{p}$cgtagacgtcctcca	cDNA synthesis of *TTHA0987* gene
P25	$_{p}$ctccttgctcccgaa	cDNA synthesis of *TTHB023* gene

Continued on next page.

TABLE S1 Continued.

P26; P27	fwd, ctggagctcttcgccaag; rev, gcccctgtgatgagcac	1st PCR for 5'-RACE for *TTHA0750* gene
P28; P29	fwd, gacgacctcctcgcccac; rev, ttgccgatgggggtccta	1st PCR for 5'-RACE for *TTHA0987* gene
P30; P31	fwd, ctacgaggccaccagcgt; rev, tcggtgaagagcttggcg	1st PCR for 5'-RACE for *TTHB023* gene
P32; P33	fwd, gtggcctgcgacatagag; rev, cttgtccttgagccgcat	2nd PCR for 5'-RACE for *TTHA0750* gene
P34; P35	fwd, ctccggcgtccccaaaga; rev, cttccacgatccaggctt	2nd PCR for 5'-RACE for *TTHA0987* gene
P36; P37	fwd, ccctggggctttccaagg; rev, gtccctcgtcgtcgttac	2nd PCR for 5'-RACE for *TTHB023* gene
P38; P39	fwd, gacctgatcctgagggggtt; rev, aggaactcctcccgcacctc	RT-PCR for *TTHB023 −018* operon

[a] fwd, forward; rev, reverse. p, phosphorylated at the 5' terminal. HTK, kanamycin-resistance marker.

TABLE S2 Genes exhibiting altered expression in the Δ*pfmR* strain, in comparison with the wild type. The expression levels in the Δ*pfmR* strain relative to those in the wild type, and the *q* values of the observed differences between the wild type and Δ*pfmR* strains, are shown. Only genes for which the *q* values are less than or equal to 0.05 are shown. *TTHA*, *TTHB*, and *TTHV* denote the genes on chromosomal DNA, pTT27, and pVV8, respectively.

Gene Name	Annotation for product	Expression (*q*)
TTHA0102	Hypothetical protein	1.477 (0.05)
TTHA0131	Hypothetical protein	0.820 (0.05)
TTHA0207	Nicotinamide nucleotide transhydrogenase, alpha subunit 2	1.456 (0.05)
TTHA0267	Oxygen-insensitive NADPH nitroreductase	1.268 (0.05)
TTHA0346	Peptidyl-prolyl cis-trans isomerase	0.622 (0.05)
TTHA0684	Probable TolQ-type transport protein	0.627 (0.05)
TTHA0750	3-oxoacyl-[acyl carrier protein] reductase	3.568 (0.05)
TTHA0751	Hypothetical protein	1.988 (0.05)
TTHA0752	Beta-lactamase family protein	1.941 (0.05)
TTHA0829	Putative acetoin utilization protein, acetoin dehydrogenase	1.585 (0.05)
TTHA0987	Beta-ketoadipyl CoA thiolase	3.851 (0.05)
TTHA1154	Hypothetical protein	0.548 (0.05)
TTHA1256	Hypothetical protein	0.718 (0.05)
TTHA1317	Immunogenic protein related protein	1.600 (0.05)
TTHA1410	Sulfite dehydrogenase cytochrome subunit SoxD	0.416 (0.05)
TTHA1908	Lysine biosynthesis enzyme LysW	1.289 (0.05)
TTHB013	Hypothetical protein	2.633 (0.05)
TTHB023	Transcriptional regulator, TetR family (PfmR)	0.003 (0.05)
TTHB125	Chromosome partitioning ATPase, ParA family	1.250 (0.05)
TTHV006 (*TTHY7063*)	Filamentation induced by cAMP protein Fic	0.015 (0.05)
TTHV007 (*TTHY7057*)	Putative adenine-specific DNA methylase	0.251 (0.05)
TTHV009 (*TTHY7058*)	HicB family protein	0.009 (0.05)
TTHV010 (*TTHY7032*)	Helix-turn-helix domain protein	0.123 (0.05)
TTHV011 (*TTHY7078*)	Hypothetical protein	0.025 (0.05)
TTHV065 (*TTHY7040*)	Transposase, IS605 OrfB family	0.012 (0.05)
TTHV066 (*TTHY7084*)	PilT protein domain protein	0.033 (0.05)
TTHV067 (*TTHY7084*)	Toxin-antitoxin system, antitoxin component, PHD family	0.033 (0.05)
TTHV034 (*TTHY7018*)	Glycerol-3-phosphate ABC transporter substrate-binding protein	0.154 (0.05)
TTHV046 (*TTHY7064*)	Hypothetical protein	0.05 (0.05)
TTHV074 (*TTHY7080*)	Hypothetical protein	0.217 (0.05)
TTHV085 (*TTHY7010*)	Xylose isomerase	0.277 (0.05)

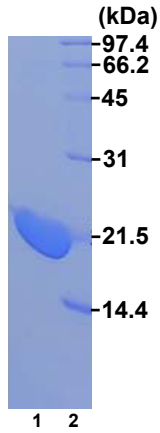

FIG. S1 Sodium dodecyl sulfate-polyacrylamide gel electrophoresis analysis of PfmR. The purified protein (4 μg) was analyzed on a 12% polyacrylamide gel, which was stained with Coomassie Brilliant Blue R-250. Lane 2, molecular mass markers.

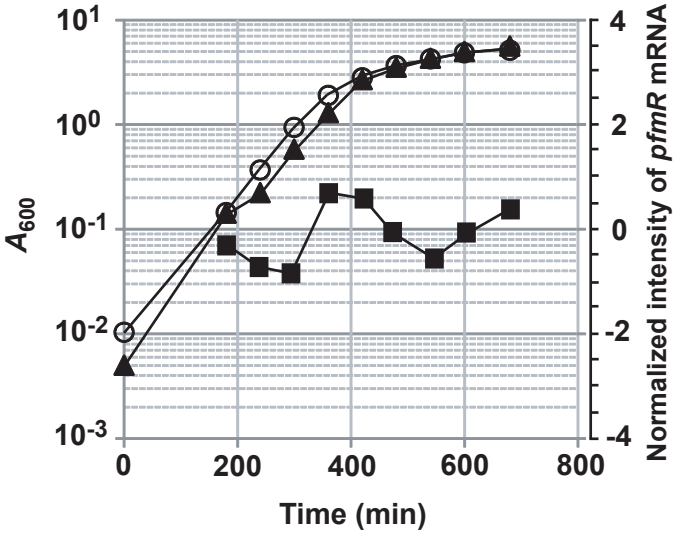

FIG. S2 Growth of *T. thermophilus* wild-type (open circles) and Δ*pfmR* (closed triangles) strains. The strains were cultured in rich medium, and the A_{600} values at the indicated times are shown. The expression of *pfmR* mRNA (Normalized intensity of *pfmR* mRNA) in the wild-type strain was investigated at the indicated times using GeneChip technology (closed squares). Each normalized intensity at the indicated times, calculated as described in the Materials and Methods in the main text, were further normalized as to the mean value of the *pfmR* intensities.

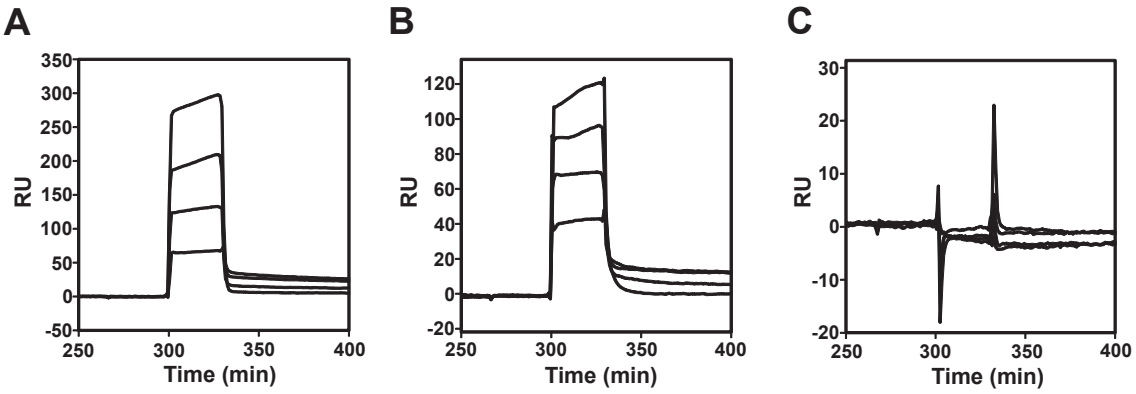

FIG. S3 BIAcore biosensor analyses of the interactions between the *T. thermophilus* TetR family transcriptional regulators and DNA. A double-stranded DNA fragment corresponding to the upstream region of the *TTHB023* gene (see text), which contains the predicted PfmR-binding site (A), that of the *TTHA0963* gene (ref. 33), which contains the predicted PaaR-binding site (B), or that of the *TTHA0890* gene (ref. 1), which contains the predicted FadR-binding site, was immobilized on the sensor chip, and then the FadR (A and B) or PaaR (C) protein was injected over the DNA surface, at concentrations of 0.4, 0.3, 0.2, and 0.1 μM dimer, as described in the text.

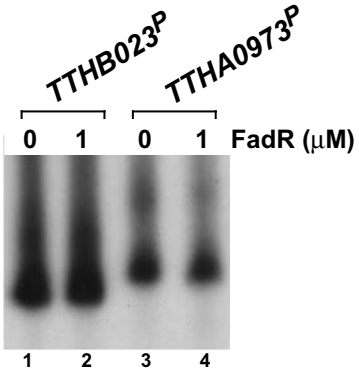

FIG. S4 Effects of *T. thermophilus* FadR on transcription *in vitro*. Run-off transcription assays were performed with templates containing the upstream sequences of the genes regulated by PfmR (*TTHB023*^{P}) (lanes 1 and 2) and PaaR (*TTHA0973*^{P}) (ref. 33) (lanes 3 and 4), in the absence (lanes 1 and 3) or presence (lanes 2 and 4) of 1 μM FadR. After the reaction, the samples were fractionated on the polyacrylamide gel, followed by autoradiography.

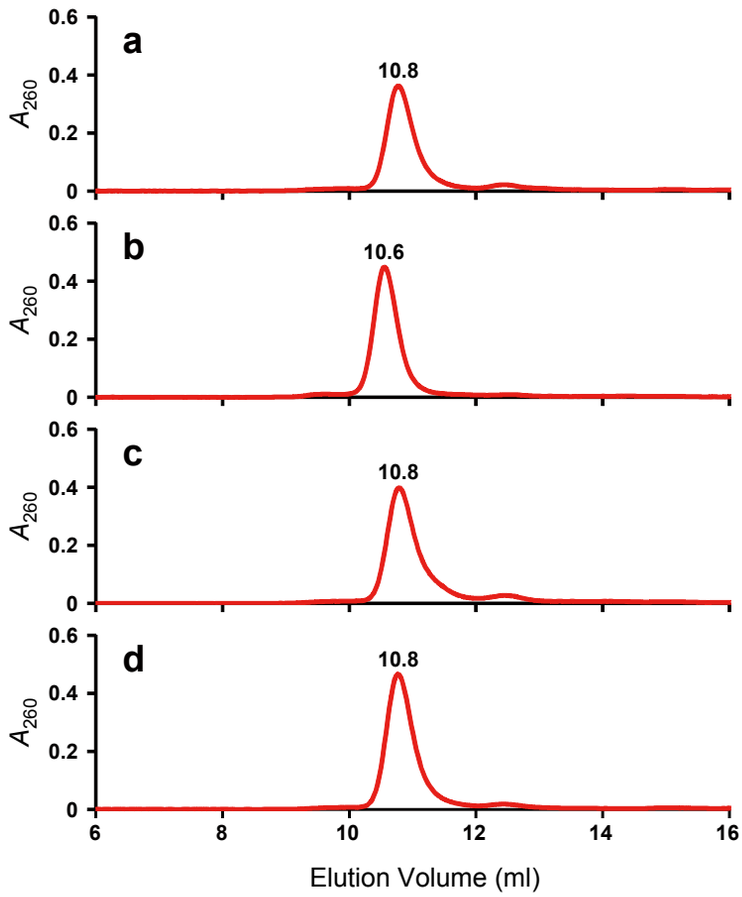

FIG. S5 Gel-filtration analysis of the DNA fragment. Elution profiles of samples (26 µl) comprising 25 µM of the 28-bp DNA fragment derived from the upstream region of the *TTHB023* gene (see text) (a), 25 µM of the 31-bp DNA fragment derived from the upstream region of the *TTHA0890* gene (ref. 1) (b), 25 µM of the 28-bp DNA fragment derived from the upstream region of the *TTHB023* gene containing the A6C/T6'G mutations (see text) (c), and 25 µM of the 28-bp DNA fragment derived from the upstream region of the *TTHB023* gene containing the C8A/G8'T mutations (see text) (d). The elution buffer was of 10 mM sodium phosphate (pH 7.0), containing 0.3 M NaCl. Relative absorbance at 260 nm is indicated. The elution volume at the top of the peak is indicated.