SUPPLEMENTAL DATA

FIGURE LEGENDS

FIGURE S1. **Marker exchange deletion and verification of the HB27 *pilQ* mutant.** (**A**) Generation of a HB27 Δ*pilQ*::*bleo* mutant. ~1000 bp of the upstream and downstream region of *pilQ* and a bleomycin cassette (*bleo*) were amplified by PCR and cloned into pBIISK using indicated restriction sites. The resulting plasmid (pBIISK-pilOWbleoChor) was linearized and transformed into HB27. Mutants where the *pilQ* gene was exchanged against the *bleo* cassette were selected on $TM^{+/Bleo}$ plates. (**B**) Verification of the Δ*pilQ*::*bleo* mutant was performed by Southern blot analyses. *Bam*HI digested genomic DNA from HB27 wild type or HB27 Δ*pilQ*::*bleo* was separated by gel electrophoresis, transferred to a nylon membrane and probed with specific DIG-labeled DNA fragments against the *pilQ* gene, *pilQ* downstream region (*TTC1018*), and *bleo* cassette.

FIGURE S2. **Purification of N-terminal truncated PilQ complexes.** Protein fractions of single purification steps were analyzed on a 3-12% polyacrylamide gradient SDS-PAGE (**A**, **C**) followed by Western blot analysis (**B**, **D**). Lane 1, crude extract; lane 2, soluble fraction; lane 3, membrane fraction; lane 4, wash fraction; lane 5, washed membranes; lane 6, membranes after Triton-X-100 solubilisation; lane 7, Triton-X-100 solubilisate; lane 8, pooled PilQ fractions of the density gradient; lane 9, pooled PilQ fractions of the Q-Sepharose; lane 10, pooled PilQ fractions of the gel filtration (Superose 6); **A** and **B**, documentation of $PilQ_{\Delta 25\text{-}64}$ complex purification; **C** and **D**, documentation of $PilQ_{\Delta 25\text{-}125}$ complex purification.

TABLE S1. Strains and plasmids used in this study.

strain or plasmid	description	source or reference
Bacteria		
***T. thermophilus* HB27**	wild type	(1)
HB27 Δ*pilQ*::*bleo*	Δ*pilQ*::*bleo*	this study
***E. coli* DH5α**	*E. coli* cloning host	(2)
Plasmids		
pWUR112	$Bleo^R$	(3)
pBIISK	Cloning vector, Amp^R	Stratagene
pBIISK-pilOWbleoChor	*pilQ* upstream and downstream region PCR product, and *bleo* cassette cloned into pBIISK	this study
pDM12	*Thermus*/*E. coli* shuttle vector, HTK, Amp^R, bc_1 promotor	(4)
pDM12-pilQhis-Q	*pilQ* cloned into pDM12, C-terminal His_6 tag	this study
pDM12-pilQ$_{\Delta 25-34}$his	pDM12 encoding $PilQ_{\Delta 25-34}$ fused to His_6-tag	this study
pDM12-pilQ$_{\Delta 25-64}$his	pDM12 encoding $PilQ_{\Delta 25-64}$ fused to His_6-tag	this study
pDM12-pilQ$_{\Delta 25-125}$his	pDM12 encoding $PilQ_{\Delta 25-125}$ fused to His_6-tag	this study
pDM12-pilQ$_{\Delta 25-207}$his	pDM12 encoding $PilQ_{\Delta 25-207}$ fused to His_6-tag	this study
pDM12-pilQ$_{\Delta 25-262}$his	pDM12 encoding $PilQ_{\Delta 25-262}$ fused to His_6-tag	this study

$Bleo^R$, heat resistant bleomycin resistance cassette; HTK, heat resistant kanamycin resistance cassette; Amp^R, ampicillin resistance cassette.

TABLE S2. Primers used in this study.

primer	sequence	added restriction site or tag	use
PilOW-for-*Sal*I	5'-ACGCGTCGACCAGGAGATTGACCGCCTCACCATAG-3'	*Sal*I	amplification of *pilQ* upstream region
PilOW-rev-*Eco*RV	5'-AGATATCCTCTTCATTGGCCACCTCCAGCTTG-3'	*Eco*RV	amplification of *pilQ* upstream region
Bleo-for-*Eco*RV	5'-AGATATCGGCGGCGCAGGCCTTCCTGG-3'	*Eco*RV	amplification of *bleo* cassette, Southern blot probe
Bleo-rev-*Pst*I	5'-AAACTGCAGCTTCCGGCTCGTATGTTGTGTGG-3'	*Pst*I	amplification of *bleo* cassette, Southern blot probe
Chor-for-*Pst*I	5'-AAACTGCAGATGCGGTTTCTCACGGCGGCGAGTC-3'	*Pst*I	amplification of *pilQ* downstream region, Southern blot probe
Chor-rev-*Not*I	5'-GAATGCGGCCGCGATGGCCGAGAGGGGCAGAGGATG-3'	*Not*I	amplification of *pilQ* downstream region, Southern blot probe
PilQ-for	5'-GCACGGTCGTCAACATCGAG-3'	None	Southern blot probe
PilQ-rev	5'-TGGCGTTGGAGAGCTGGTAG-3'	None	Southern blot probe
PilQ-for-*Nde*I	5'-GGGAATTCCATATGAAGAGCGCGTGGATCCGTGCGG-3'	*Nde*I	amplification of *pilQ*
PilQ-rev-*Not*I	5'-ATAAGAATGCGGCCGCTCAGTGGTGGTGGTGGTGGTGGGGGTTCGCCGAGGCCGCCTCCTTG-3'	*Not*I, stop codon, 6x his-tag	amplification of *pilQ*
pDM12PilQ-rev	5'-GCTCCCCGCAAGGGCAAAAGC-3'	None	SDM
pDM12PilQ-for-A	5'-GTGAACCTCAAGGTCTCGGAATC-3'	None	SDM
pDM12PilQ-for-B	5'-CTCCAGCCCCTCATCTACCG-3'	None	SDM
pDM12PilQ-for-D	5'-GTGGTGGCCCCCACCCAGG-3'	None	SDM
pDM12PilQ-for-E	5'-GAAGGGGGTAGGCTCAAGGC-3'	None	SDM
pDM12PilQ-for-F	5'-TTCCCCGACCTCCTCGCCTTC-3'	None	SDM

underlined bp indicate restriction sites *Sal*I, *Eco*RV, *Pst*I, *Nde*I, *Not*I; SDM, Phusion-site directed mutagenesis

Supplementary Information: Mass spectrometry

Samples for protein m/z determination were pre-incubated with TFA (typically 5 µg of protein in 3µl was incubated with 3 µl TFA at 90°C for 5 min) and purified using C4-ZipTips (Millipore Corporation, Billerica, USA) using standard protocols. Eluates were mixed in a 1:1 (v/v) ratio with matrix (DHAP, 15 mg/ml in 75% ethanol in 20 mM sodium citrate, Bruker Daltonics, Bremen, Germany) and spotted on ground steel target plates (Bruker Daltonics, Bremen, Germany). MALDI mass spectra were recorded in a Bruker Autoflex III Smartbeam mass spectrometer with a mass range of 30-100 kDa. Detection was optimized for high m/z values and calibrated using calibration standards in this mass range (Protein high molecular weight calibration standard 2, Bruker Daltonics, Bremen).

Protein samples for sequence characterization were submitted to SDS-PAGE, and peptide mass fingerprinting analyses were performed on reduced and alkylated, trypsin- and chymotrypsin-digested samples prepared by standard mass spectrometry protocols (Proteoextract Digestion kit, CalBiochem).

Proteolytic digests were loaded using a nano-HPLC (Proxeon easy-nLC) on reverse phase columns (trapping column: particle size 5 µm, C18, L=20 mm; analytical column: particle size 5 µm, C18, L=15 cm; NanoSeparations, Nieuwkoop, The Netherlands), and eluted in gradients of water (0.1% formic acid, buffer A) and acetonitrile (0.1% formic acid, buffer B) Typically, gradients were ramped from 5% to 65% B in 50 minutes at flow rates of 300 nl/min (extended gradients: 5% to 65% B in 100 minutes). Peptides eluting from the column were ionised online using a Bruker Apollo ESI-source with a nanoSprayer emitter and analysed in a quadrupole time-of-flight mass spectrometer (Bruker maXis). Mass spectra were acquired over the mass range 50-2200 m/z, and sequence information was acquired by computer-controlled, data-dependent automated switching to MS/MS using collision energies based on mass and charge state of the candidate ions.

The data sets were processed using a standard proteomics script with the software Bruker DataAnalysis 4.0 Service Pack 1 Build 253 and exported as mascot generic files. Spectra were internally recalibrated on autoproteolytic trypsin fragments when applicable.
Proteins were identified by matching the derived mass lists against the NCBInr database (downloaded from http://www.ncbi.nlm.nih.gov/) on a local Mascot server (Version 2.2.2, Matrix Science, UK). In general, a mass tolerance ± 0.05 Da for parent ion and fragment spectra, two missed cleavages, oxidation of Met and fixed modification of carbamidomethyl cysteine were selected as matching parameters in the search program.

These data and the results from re-sequencing the open reading frames were analyzed and validated using the BioTools 3.1 software package (build 2.22, Bruker Daltonics). The proteomics data associated with this manuscript were uploaded to the PRIDE online repository. Anonymous reviewer access is granted upon request.

References

1. Oshima, T., and Imahori, K. (1974) *Int. J. Syst. Bacteriol.* **24**, 102-112
2. Hanahan, D. (1983) *J. Mol. Biol.* **166**, 557-580
3. Brouns, S. J., Wu, H., Akerboom, J., Turnbull, A. P., de Vos, W. M., and van der Oost, J. (2005) *J. Biol. Chem.* **280**, 11422-11431
4. Werner, C., Richter, O. M. H., and Ludwig, B. (2010) *J. Bacteriol.* **192**, 4712-4719