Supporting Information

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SI Materials and Methods

Construction of Plasmids. Plasmids, primers, and strains used and constructed in this study are listed in Tables S2–S4. For cloning, *Escherichia coli* strains DH5α and Top10 were used. Unless otherwise indicated, PCR was performed on either chromosomal DNA of *Neisseria gonorrhoeae* strain MS11 or *Myxococcus xanthus* strain DK1622. To construct pSJ082, T4P secretin-associated protein (tsaP) (annotated as NGFG_01788 in *N. gonorrhoeae* strain MS11) was amplified by PCR using primers 592 and 593, digested with KpnI and BamHI, and ligated into pIDN3. To construct pKS001, $tsaP_{NG}$ was amplified by PCR using primers 1001 and 1002, digested with KpnI and EcoRI, and ligated into pIDN3. To construct pKS007, $tsaP_{NG}$ was amplified by PCR using primers 1058 and 1057, digested with NdeI and HindIII, and cloned into pSJ023. To construct pAW001, $tsaP_{NG}$ was amplified by PCR using primers 1061 and 1052, digested with NdeI and XhoI, and ligated into pET20b(+). pIMB1 was constructed by cloning overlap PCR product of the two PCR fragments generated with the omxan_3001-A and omxan_3001-B primers and the omxan_3001-C and omxan_3001-D primers into the HindIII and EcoRI sites of pBJ114. To construct pIMB2, the $tsaP_{MX}$ gene (annotated as Mxan_3001 in *M. xanthus* strain DK1622) was amplified by PCR with the primers omxan_3001-1 and omxan_3001-2, digested with XbaI and HindIII, and cloned into pSW105. To create pIMB3, the *pilA* promoter and $tsaP_{MX}$ were amplified from pIMB2 using the primers omxan_3001-3 and omxan_3001-4. The PCR product was digested with EcoRI and BamHI, and cloned into the pKA28 vector. To create pIMB5, $tsaP_{MX}$ lacking the first 21 bp was amplified with the omxan_3001-7 and omxan_3001-8 primers, digested with BamHI and HindIII, and cloned into the pET45b(+) vector.

Construction of *N. gonorrhoeae* Strains. *N. gonorrhoeae* mutants were created by homologous recombination into the chromosome. Either plasmid DNA or PCR products were introduced by natural transformation or electroporation as described previously (1). MS11 was used as the WT strain. To generate SJ004-MS, in which a sequence encoding a His_8-tag was inserted between P154 and F155 of the small basic repeat region of PilQ, two PCR products were generated with primers 552 and 572 and primers 573 and 553. These products were mixed and used as a template for a third PCR using primers 552 and 553. This PCR product was transformed to strain MS11. To generate strain SJ082-MS, in which *tsaP* is disrupted by insertion of plasmid pIDN3, MS11 was transformed with pSJ082. To generate strain KS035, in which the *tsaP* deletion in strain SJ082-MS is complemented by a copy of *tsaP* behind the *lac* promoter of plasmid pSJ023 inserted between the *lctP* and *aspC* genes, SJ082-MS was transformed with pKS007. All chromosomal mutations were confirmed by DNA sequence analysis.

Construction of *M. xanthus* Strains. For all experiments with *M. xanthus*, DK1622 was used as WT and all *M. xanthus* strains used are derivatives of DK1622. Plasmids were introduced into *M. xanthus* by electroporation. SA6011, which contains an in-frame deletion of *tsaP* from positions 31–1,176 relative to the start codon, was generated by transformation of DK1622 with pIMB1 using standard techniques (2). *M. xanthus* strains SA6014 and SA6013, in which the $tsaP_{MX}$ deletion is complemented either by $tsaP_{MX}$ or by a C-terminal fusion of mCherrry to $tsaP_{MX}$ under control of the *pilA* promoter, were generated by electroporation of SA6011 with pIMB2 and pIMB3, respectively, followed by integration of the two plasmids at the Mx8 *attB* site. The C-terminal fusion of mCherry to $tsaP_{MX}$ under control of the *pilA* promoter was introduced in different strains by electroporation with plasmid pIMB3. C-terminal fusions of PilO, PilP, and PilQ to superfolder GFP (sfGFP) under control of the *pilA* promoter were introduced in the Δ*tsaP* strain by electroporation with the plasmids pSC106, pSC102, and pSC120, respectively. An N-terminal fusion of YFP to PilT under control of the *pilA* promoter was introduced in the Δ*tsaP* strain by electroporation with the plasmid pIB75. All strains constructed were confirmed by PCR.

Growth. *E. coli* stains were grown at 37 °C in either LB or Luria agar plates. When required, erythromycin (450 μg/mL), ampicillin (100 μg/mL), or isopropyl-β-D-thiogalactoside (IPTG; 0.5 M) was added. *N. gonorrhoeae* strains were grown in gonoccocal base medium liquid (GCBL) medium with 0.042% sodium bicarbonate and Kellogg's supplements (3) or on gonococcal base (GCB) plates containing Kellogg's supplements at 37 °C in 5% (vol/vol) CO_2. When required, erythromycin (10 μg/mL), chloramphenicol (10 μg/mL), or IPTG (1 mM) was added. *M. xanthus* strains were grown at 32 °C in 1% Casitone-Tris (CTT) broth (4) or on CTT agar plates supplemented with 1.5% (wt/vol) agar. Kanamycin (50 μg/mL) or oxytetracycline (10 μg/mL) was added when appropriate.

Detailed Purification of TsaP. *E. coli* BL21 star (DE3) cells transformed with pAW001 were grown to an OD_{600} of 0.5 in LB at 37 °C and induced with 0.5 mM IPTG. After incubation for another 3 h, cells were harvested by centrifugation at 4 °C for 10 min at 7,500 × *g*. Cells were resuspended in 10 mL of buffer B [100 mM NaCl, 15 mM imidazole, 10% (wt/vol) glycerol, 50 mM Hepes (pH 7.5)] containing Protease Inhibitor Mixture (Roche) and 10 μg/mL DNase I, and then lysed by three passages through a French press at 1,000 psi. The suspension was centrifuged at 4 °C for 10 min at 12,000 × *g*, followed by ultracentrifugation for 30 min at 180,000 × *g*. The supernatant was loaded on a HiTrap Chelating HP column (GE Healthcare) equilibrated with buffer B. After washing the column with buffer B containing 20, 40, and 50 mM imidazole, TsaP was eluted with a gradient from 50–400 mM imidazole in buffer B. Fractions containing TsaP were collected. These fractions were applied to a Superdex 75 HiLoad 16/60 column equilibrated with buffer C [100 mM NaCl, 50 mM Hepes (pH 7.5)]. Fractions containing TsaP were collected and frozen.

Detailed Purification of His6-$TsaP_{MX}$Δ7 Under Denaturing Conditions. *E. coli* Rosetta 2 (DE3) cells transformed with plasmid pIMB5 were grown to an OD_{600} of 0.5 in LB at 37 °C and induced with 1 mM IPTG at 18 °C overnight. His_6-$TsaP_{MX}$Δ7 was purified from inclusion bodies under denaturing conditions on an Ni^{2+}-nitrilotriacetic acid-agarose column (Qiagen) using buffer B [8 M urea, 10 mM Tris, 100 mM NaH_2PO_4 (pH 8.0)] as described by the manufacturer. The bound protein was eluted with buffer E containing 8 M urea, 10 mM Tris, and 100 mM NaH_2PO_4 (pH 4.5), and then dialyzed against 300 mM NaCl, 10 mM imidazole, and 50 mM NaH_2PO_4 (pH 8.0).

Peptidoglycan Isolation, Binding, and Zymography. Murein sacculi of *N. gonorrhoeae* cells were purified as described previously (5) from 3 L of an exponentially growing culture. The presence of sacculi was confirmed by EM. To test for binding to peptidoglycan (PG), 5 μg of purified *N. gonorrhoeae* TsaP or 5 μg of *E. coli* exonuclease I was incubated with or without 1 mg of PG in a volume of 150 μL for 1 h at 15 °C in protein purification buffer C [100 mM NaCl, 50 mM Hepes (pH 7.5)]. Samples were spun

down in an airfuge (Beckman Coulter) at 20 pounds per square inch gage (psig) for 10 min. The supernatant (unbound fraction) was collected, and the pellet was resuspended in protein purification buffer C. The samples were spun down again at 20 psig for 10 min. The supernatant (wash fraction) was collected, and the pellet fraction was resuspended in 4% (wt/vol) SDS in buffer C, incubated for 2 h at 15 °C, and centrifuged for 5 min at 15 psig. The supernatant was collected (bound fraction). All fractions were precipitated with trichloroacetic acid and analyzed by immunoblotting. To test for PG hydrolysis activity of TsaP, 5 μg of purified TsaP was loaded on 12% SDS/PAGE gels containing 0.04% PG and zymography was performed essentially as described (5). Five micrograms of BSA and 5 μg of lysozyme and mutanolysin were loaded as negative and positive controls, respectively.

1. Dillard JP (2011) Genetic manipulation of Neisseria gonorrhoeae. *Curr Protoc Microbiol* Chapter 4:Unit4A2. (Wiley, Hoboken, NJ), pp 1–24.
2. Shi X, et al. (2008) Bioinformatics and experimental analysis of proteins of two-component systems in *Myxococcus xanthus*. *J Bacteriol* 190(2):613–624.
3. Nicolson IJ, Perry AC, Heckels JE, Saunders JR (1987) Genetic analysis of variant pilin genes from *Neisseria gonorrhoeae* P9 cloned in *Escherichia coli:* Physical and immunological properties of encoded pilins. *J Gen Microbiol* 133(3):553–561.
4. Hodgkin J, Kaiser D (1977) Cell-to-cell stimulation of movement in nonmotile mutants of Myxococcus. *Proc Natl Acad Sci USA* 74(7):2938–2942.
5. Möll A, Schlimpert S, Briegel A, Jensen GJ, Thanbichler M (2010) DipM, a new factor required for peptidoglycan remodelling during cell division in *Caulobacter crescentus*. *Mol Microbiol* 77(1):90–107.

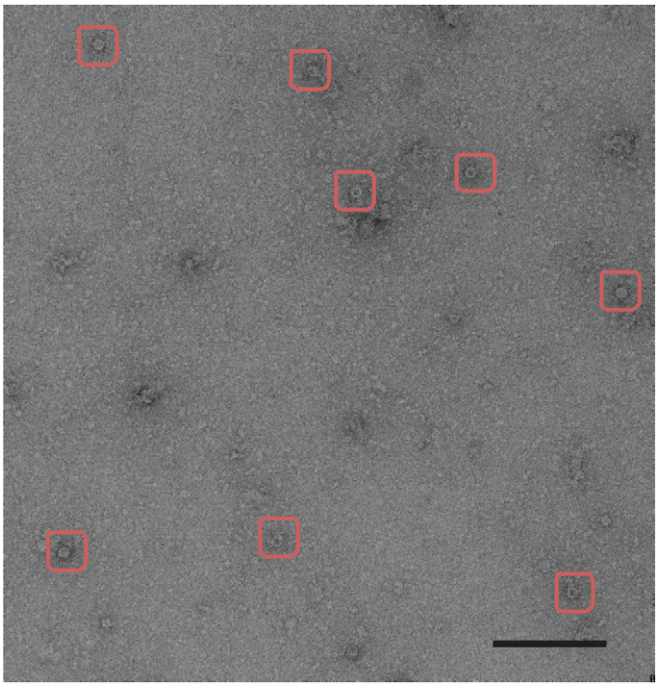

Fig. S1. Analysis of purified PilQ by EM. Elution fractions of the PilQ purification were applied to carbon-coated copper grids and negatively stained with 2% (wt/vol) uranyl acetate. PilQ particles are indicated by the red boxes. (Scale bar: 100 nm.)

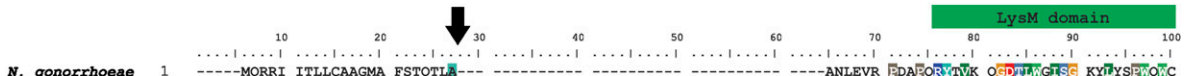

Fig. S2. Alignment of TsaP homologs of different organisms. TsaP homologs described in Table S1 were aligned. Colored residues are <50% conserved. The putative signal sequence cleavage site is indicated with a black arrow, whereas the LysM domain is indicated with a green box. Residues (G40, D41, T42, N70, and G77) that are highly conserved in LysM domains are indicated in bold below the alignment.

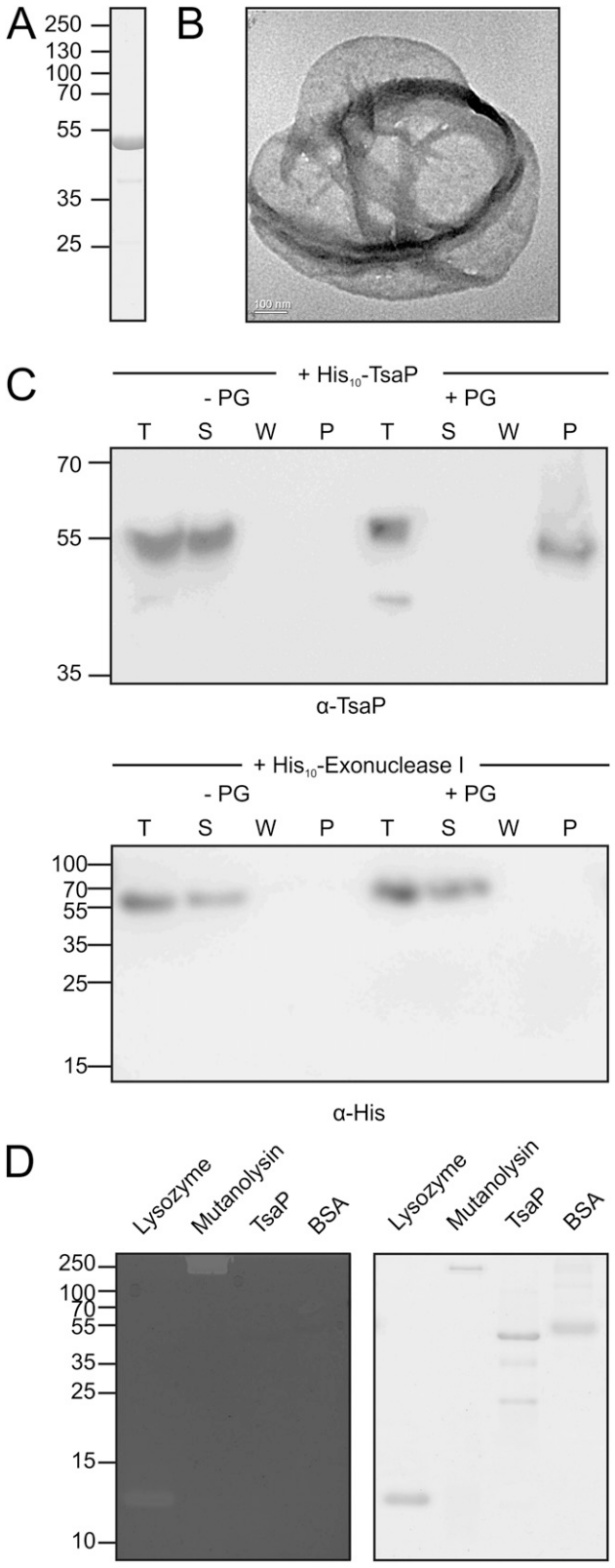

Fig. S3. Characterization of binding of TsaP to isolated PG sacculi. (*A*) TsaP was overexpressed and purified from *E. coli*. Fractions eluted from the size exclusion column at a size corresponding to the monomer were analyzed by SDS/PAGE and Coomassie staining. (*B*) PG sacculi were isolated from *N. gonorrhoeae* and analyzed by EM. (*C*) To test for binding of TsaP to PG, 5 μg of TsaP was incubated without (*Upper*, −PG) or with (*Upper*, +PG) 1 mg of PG. Samples (total, T) were centrifuged, the supernatant was collected (supernatant, S), and the pellet was resuspended. The samples were centrifuged again, the supernatant (wash, W) was collected, and the pellet fraction (Pellet, P) was resuspended. The different fractions were analyzed by immunoblotting using α-TsaP antibodies. As a control, 5 μg of purified His_{10}-tagged exonuclease I was incubated without (*Lower*, −PG) or with (*Lower*, +PG) 1 mg of PG, treated as described above, and

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analyzed using α-His antibodies. To test for PG hydrolysis, zymography was performed. (*D*) Lysozyme, mutanolysin, TsaP, and BSA (5 μg each) were applied to SDS gels containing purified murein sacculi. (*Left*) Proteins were stained with Coomassie blue. (*Right*) Second gel was incubated in renaturation buffer to allow for refolding of the proteins, and PG hydrolysis was detected by staining of sacculi with methylene blue. Clear zones of hydrolysis are observed for mutanolysin and lysozyme but not for BSA and TsaP. The hydrolysis zone of mutanolysin was observed at a height comparable to proteins with a molecular weight of ~250 kDa. Most likely, mutanolysin is not completely unfolded during the SDS/PAGE and is retarded by binding to the murein during electrophoresis, an effect that has been seen for other proteins before (1). Because the high level of O-acetylation of *N. gonorrhoeae* murein inhibits the activity of lysozyme (2), the specific activity of lysozyme was lower than the activity of mutanolysin.

1. Juarez ZE, Stinson MW (1999) An extracellular protease of *Streptococcus gordonii* hydrolyzes type IV collagen and collagen analogues. *Infect Immun* 67(1):271–278.
2. Blundell JK, Perkins HR (1985) The peptidoglycan of *Neisseria gonorrhoeae*, with or without O-acetyl groups, contains anhydro-muramyl residues. *J Gen Microbiol* 131(12):3397–3400.

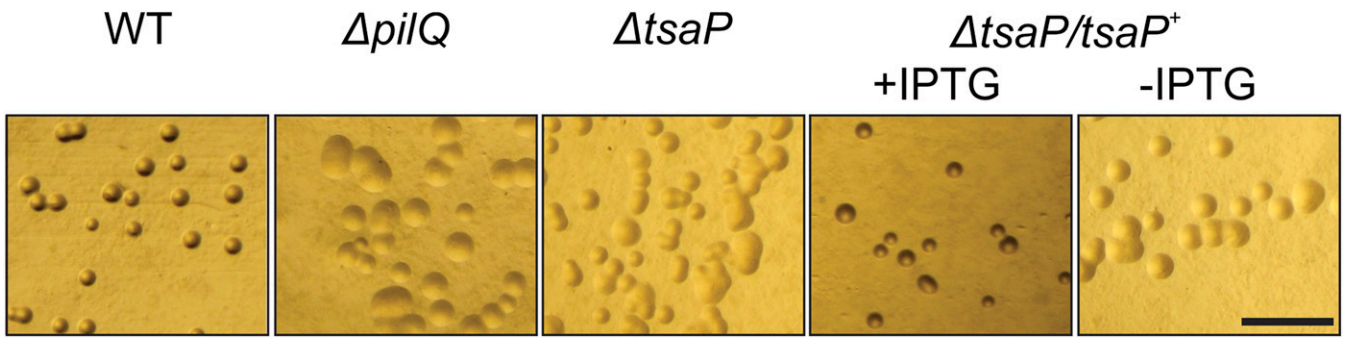

Fig. S4. TsaP is important for type 4 pili (T4P)-dependent colony morphology. The indicated *N. gonorrhoeae* strains were incubated at 37 °C for 24 h on GCB plates. (Scale bar: 1 cm.)

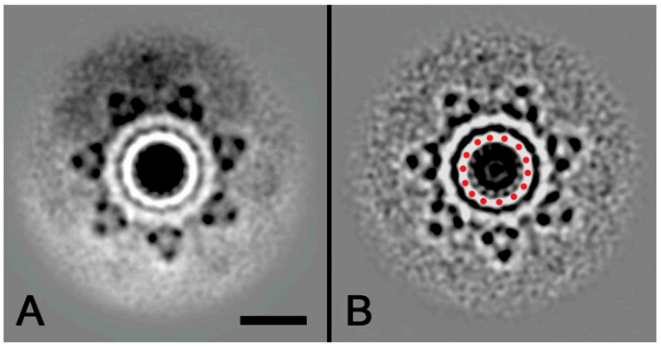

Fig. S5. Projection maps of single-particle EM analysis of the PilQ complex from the *N. gonorrhoeae ΔtsaP/tsaP*$^{+}$ strain. (*A*) Two-dimensional map of the *ΔtsaP/tsaP*$^{+}$ particle seen in a slightly tilted top-view position. A small protein domain is visible inside the inner rings, especially in the lower half. It is present in 14 copies, but some copies in the upper half are partly invisible because of partial overlap with the main body of the ring, which is the effect of tilt on the carbon support film of most of the particles. (*B*) Fine details become stronger after imposing a high-pass filter on the image in such a way that all waves with a frequency representing wavelengths lower than 1.5 nm have been suppressed by 50%. The red dots mark places with a wider part of the inner rings to which the small protein domains are connected. (Scale bar: 10 nm.)

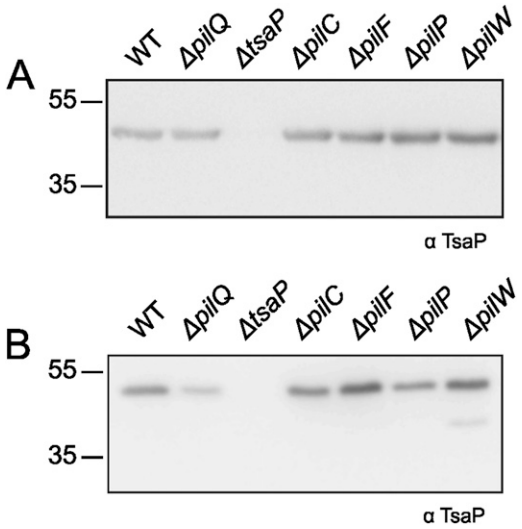

Fig. S6. TsaP levels are reduced in membranes of the *ΔpilQ* strain but not in the membranes of the *ΔpilC, ΔpilF, ΔpilP*, and *ΔpilW* strains. (*A*) Immunoblot analysis of equal amounts of total cell extracts of the *N. gonorrhoeae* WT, *ΔpilQ*, *ΔtsaP, ΔpilC, ΔpilF, ΔpilP*, and *ΔpilW* strains using α-TsaP antibodies. (*B*) Immunoblot analysis of membranes isolated from the *N. gonorrhoeae* WT, *ΔpilQ*, *ΔtsaP*, *ΔpilC, ΔpilF, ΔpilP*, and *ΔpilW* strains using α-TsaP antibodies.

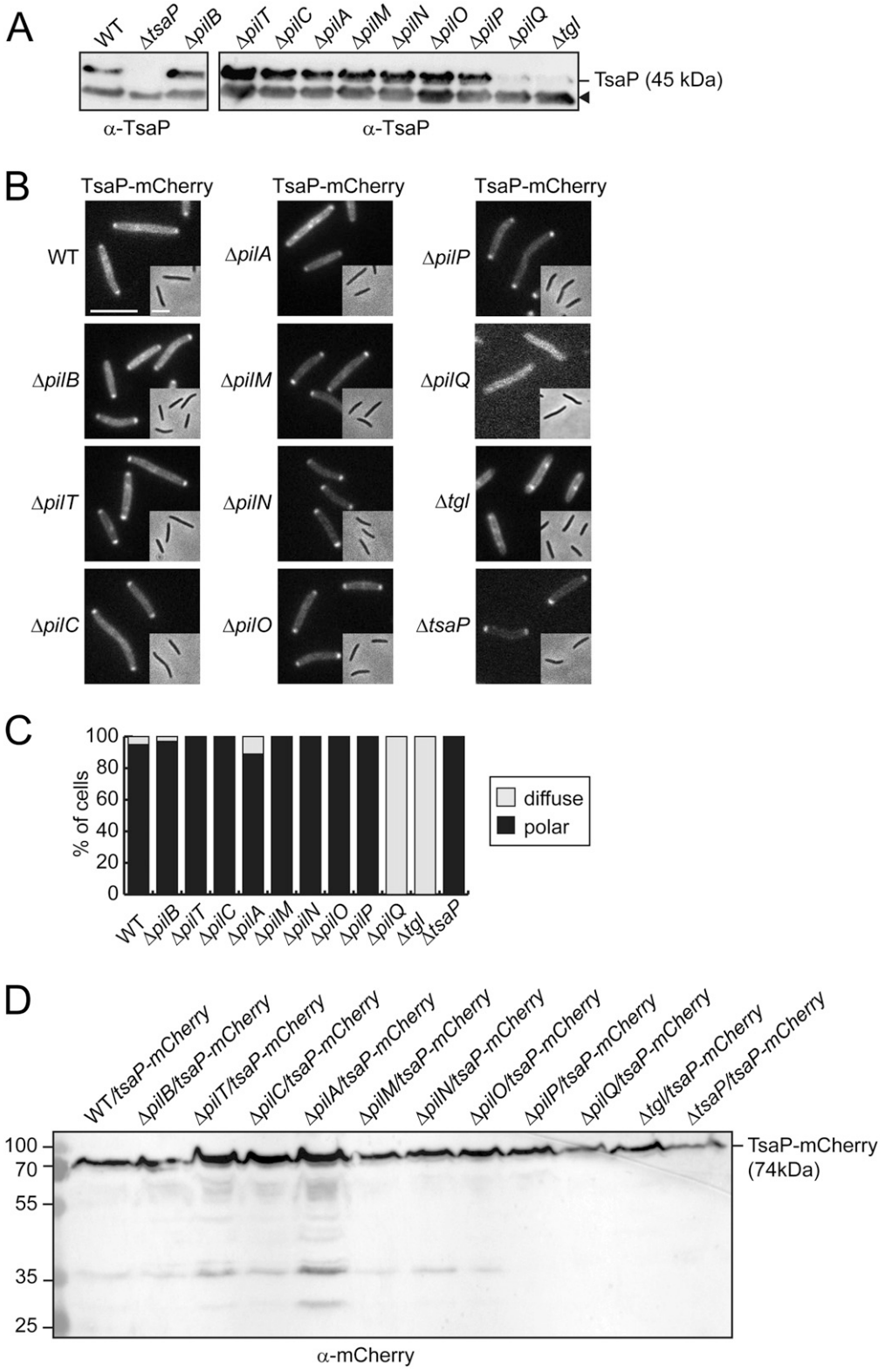

Fig. S7. Polar localization of $TsaP_{MX}$ specifically depends on $PilQ_{MX}$ and Tgl_{MX}. (*A*) TsaP protein levels in WT and the indicated in-frame deletion mutants. Equal amounts of total cell extracts of the indicated strains were separated by SDS/PAGE and analyzed by immunoblotting with antibodies directed against TsaP. A nonspecific band present in all samples is indicated by the black triangle. (*B*) Localization of $TsaP_{MX}$-mCherry in WT and the indicated in-frame deletion mutants. Cells from exponentially growing cultures of the indicated genotypes were transferred to a 1.5% (wt/vol) agar pad on a microscope slide and imaged by fluorescence microscopy. (*Insets*) Corresponding phase-contrast images. Note that the *ΔpilQ* strain is also included for comparison. (Scale bar for main figure and *Inset*: 5 μm.) (*C*) Patterns of fluorescence signals were grouped into two categories. The histogram illustrates the distribution of localization patterns of $TsaP_{MX}$-mCherry in the indicated in-frame deletion mutants ($n = 90–250$). (*D*) Immunoblot analysis of the accumulation of $TsaP_{MX}$-mCherry. Equal amounts of total cell extracts of the indicated strains were separated by SDS/PAGE and analyzed by immunoblotting using α-mCherry antibodies. Positions of $TsaP_{MX}$-mCherry (*Right*) and molecular size markers (*Left*) in kilodaltons are indicated.

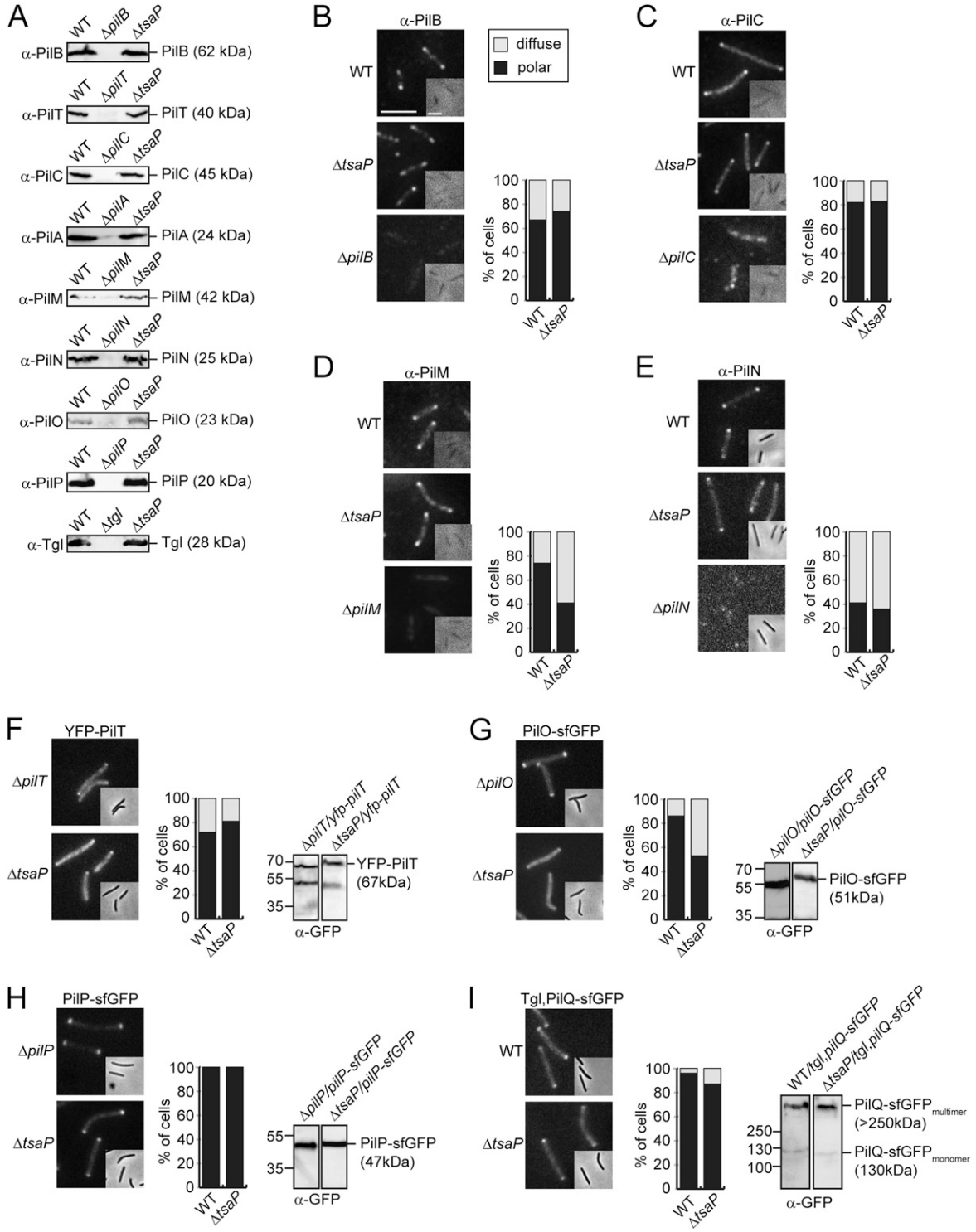

Fig. S8. Accumulation and localization of *M. xanthus* proteins involved in T4P are independent of $TsaP_{MX}$. (*A*) Immunoblot analyses of the accumulation of different *M. xanthus* proteins involved in T4P assembly in the $\Delta tsaP_{MX}$ mutant. Immunoblotting was performed as described in Fig. S7*C* using the indicated antibodies. Detected proteins with molecular sizes are indicated on the right. (*B–E*) Localization of $PilB_{MX}$, $PilC_{MX}$, $PilM_{MX}$, and $PilN_{MX}$ was determined by immunofluorescence microscopy with specific antibodies. (*Insets*) Corresponding phase-contrast images. (Scale bar for main figure and *Inset*: 5 μm.) The histograms illustrate the distribution of localization patterns (n = 150–330). Black and gray bars indicate polar and diffuse localization, respectively. (*F–I*) Localization of $PilT_{MX}$, $PilO_{MX}$, $PilP_{MX}$, and $PilQ_{MX}$ in the $\Delta tsaP_{MX}$ mutant was investigated using fluorescent fusions as described in Fig. S7*A*. (*Insets*) Corresponding phase-contrast images. (Scale bar for main figure and *Inset*: 5 μm.) The histograms illustrate the distribution of localization patterns (n = 60–250). Black and gray bars indicate polar and diffuse localization, respectively. (*Right*) Immunoblot analyses of the accumulation of fluorescently tagged proteins are shown. Relevant proteins and molecular size markers in kilodaltons are indicated.

Table S1. Nomenclature of TsaP homologs and ATPase and secretin proteins of type IV pili assembly systems and type 2 secretion systems of different organisms

	Type IV pili				Type 2 secretion system	
Organism	Assembly ATPase	Secretin	Retraction ATPase	TsaP homolog	Secretion ATPase	Secretin
Neisseria gonorrhoeae[a]	PilF	PilQ	PilT	NGFG_01788		
Neisseria meningitdis[b]	PilF	PilQ	PilT, PilU	NMC0101		
Ralstonia solanacearum[c]	PilF	PilQ	PilT, PilU	RSc0069		
Methylobacillus flagellatus[d]	PilF	PilQ	PilT, PilU	Mfla_0188		
Azoarcus spp.[e]	PilF	PilQ	PilT, PilU	azo0098		
Methylococcus capsulatus[f]	PilB	PilQ	PilT, PilU	MCA2842		
Dichelobacter nodosus[g]	PilB	PilQ	PilT, PilU	DNO_0155		
enteropathogenic Escherichia coli[h]	HofB	HofQ	—	—		
Plasmid R64[i]	PilQ	PilN	—	—		
Aggregatibacter actinomycetemcomitans[j]	TadA	RcpA	—	—		
Shewanella putefaciens[k]	PilB	PilQ	PilT, PilU	Sputcn32_0025	GspE	GspD
Pseudomonas aeruginosa[l]	PilB	PilQ	PilT, PilU	PA0020	XcpR	XcpQ
Legionella pneumophila[m]	PilB	PilQ	PilT	lpg2596	LspE	LspD
Vibrio cholerae[n]	PilB	PilQ	PilT, PilU	VC0047	GspE	GspD
Xanthomonas campestris[o]	PilB	PilQ	PilT, PilU	XCC3750	XpsE	XpsD
Myxococcus xanthus[p]	PilB	PilQ	PilT	MXAN_3001	GspE	GspD
Klebsiella oxytoca[q]	—	—	—	—	PulE	PulD
Yersinia enterocolitica[r]	—	—	—	—	GspE	GspD

[a]*Neisseria gonorrhoeae* MS11.
[b]*Neisseria meningitidis* Fam18.
[c]*Ralstonia solanacearum* GMI1000.
[d]*Methylobacillus flagellatus* KT.
[f]*Azoarcus* sp. BH72.
[g]*Methylococcus capsulatus str. Bath*.
[h]*Dichelobacter nodosus* VCS1703A.
[i]*Escherichia coli O104:H4 str. C227-11*.
[j]*Aggregatibacter actinomycetemcomitans* D11S-1.
[k]*Shewanella putrefaciens* CN-32.
[l]*Pseudomonas aeruginosa* PAO1.
[m]*Legionella pneumophila subsp. pneumophila str*. Philadelphia I.
[n]*Vibrio cholerae O1 biovar El Tor str*. N16961.
[o]*Xanthomonas campestris pv. Campestris str*. American Type Culture Collection 33913.
[p]*Myxococcus xanthus* DK1612.
[q]*Klebsiella oxytoca* KCTC 1686.
[r]*Yersinia enterocolitica subsp. enterocolitica* 8081.

Table S2. Plasmids used in this work

Plasmid	Relevant characteristics	Source
pET20b(+)	Expression vector, T7 promoter; Amp^R	Merck Millipore
pET45b(+)	Expression vector, T7 promoter, N-terminal His_6-tag; Amp^R	Merck Millipore
pIDN3	Insertion duplication mutagenesis vector for *N. gonorrhoeae*, Erm^R	1
pSW105	Vector with Mx8 *attP* site and *pilA* promoter, Km^R	S. Weiss (Max Planck Institute for Terrestrial Microbiology)
pSWU30	Vector with Mx8 *attP* site, Tet^R	2
pKA28	pSWU30 with *pomZ*-mCherry, Mx8 *attP*;Tet^R	3
pBJ114	Vector for in-frame deletion constructs, Km^R	4
pSJ023	N-terminal One-STrEP-tagged *ssb* gene cloned in pKH37 vector, Cm^R	5
pIB75	pSWU30-P_{pilA}-*yfp-pilT*, Tet^R	6
pSC102	pSC101-P_{pilA}-*pilP*, Tet^R	7
pSC106	pSC101-P_{pilA}-*pilO*, Tet^R	7
pSC120	pSC101-P_{pilA}-*tgl-pilQ*, Tet^R	7
pSJ082	pIDN3-*tsaP*, Erm^R	This study
pKS007	pSJ023-*tsaP*, Cm^R	This study
pAW001	Vector for overexpression of 10× His-tagged TsaP, Amp^R	This study
pIMB1	pBJ114-$tsaP_{MX}$ in-frame deletion, Km^R	This study
pIMB2	pSW105-P_{pilA}-$tsaP_{MX}$, Km^R	This study
pIMB3	pSWU30-P_{pilA}-$tsaP_{MX}$-mCherry, Tet^R	This study
pIMB5	pET45b(+)-$tsaP_{MX\Delta 7}$, Amp^R	This study

1. Hamilton HL, Domínguez NM, Schwartz KJ, Hackett KT, Dillard JP (2005) *Neisseria gonorrhoeae* secretes chromosomal DNA via a novel type IV secretion system. *Mol Microbiol* 55(6): 1704–1721.
2. Jakovljevic V, Leonardy S, Hoppert M, Søgaard-Andersen L (2008) PilB and PilT are ATPases acting antagonistically in type IV pilus function in *Myxococcus xanthus*. *J Bacteriol* 190(7): 2411–2421.
3. Treuner-Lange A, et al. (2013) PomZ, a ParA-like protein, regulates Z-ring formation and cell division in *Myxococcus xanthus*. *Mol Microbiol* 87(2):235–253.
4. Julien B, Kaiser AD, Garza A (2000) Spatial control of cell differentiation in *Myxococcus xanthus*. *Proc Natl Acad Sci USA* 97(16):9098–9103.
5. Jain S, et al. (2012) Characterization of the single stranded DNA binding protein SsbB encoded in the Gonoccocal Genetic Island. *PLoS ONE* 7(4):e35285.
6. Bulyha I, et al. (2009) Regulation of the type IV pili molecular machine by dynamic localization of two motor proteins. *Mol Microbiol* 74(3):691–706.
7. Friedrich C, Bulyha I, Sogaard-Andersen L (2014) Outside-In Assembly Pathway of the Type IV Pilus System in Myxococcus xanthus. *J Bacteriol* 196(2):378–390.

Table S3. Primers used in this work

Name	Nucleotide sequence (5′–3′)[a]
552	ATGCCGTCTGAAGGTCTCTTTGTCGCAACCGC
553	TTCAGACGGCATCGCCTGACTCGAGGTTTTGTT
572	ATGGTGGTGATGATGGTGATGGCTACCACTTCC TGAACCACCACTGCCCGGTGCGGCAGCCTGTTGTTTTGCCGGTG
573	ATCACCATCATCACCACCATCACGGCTCCGGCTCAAGTGGTGGCAGC GGATTTACCGAGTCCGTAGTATCCG
592	gagtggtaccTACAGCCCGTGGCAATGGTG
593	catcggATCcAAGGATGGGTGCGGGTGTAG
1052	cggcgtatacatatgcaccatcaccaccatcatcaccatcaccacGCAAATCTGGAGGTGCGCCC
1058	gcgcgccaagcttTTATTGGAAAGGGTCGGAATCG
1057	gcgcccaTATGCAACGTCGTATTATAACCCTGCTCTGCGCGGCAGGTATGGCATTCTC
1061	gcggcgctcgaGTTATTGGAAAGGGTCGGAATC
Omxan3001-A	atcggaagcttACGAGCAGATGGAGATGT
Omxan3001-B	cgcgcccgcCATCAGCGAGGTGAGAAT
Omxan3001-C	tcgctgatgGCGGGCGCGTCGCCCTCG
Omxan3001-D	atcgggaattcAGGAAGCCCTGATCCGGA
Omxan3001-1	atcggtctagaATGCGCTCCCGGATTCTC
Omxan3001-2	atcggaagcttTCAGCGGCTGGCCGAGGG
Omxan3001-3	atcgggaattcGCGGCGTTGAACGAGGGG
Omxan3001-4	atcggggatccGCGGCTGGCCGAGGGCGA
Omxan3001-7	atcggggatccGATGTCGCTGATGTTGAGCCTC
Omxan3001-8	atcggaagcttTCAGCGGCTGGCCGAGGG

[a]Nucleotides that align with the targeted sequence are depicted in capitals.

Table S4. Strains used in this work

Strain	Relevant characteristics[a]	Source
E. coli		
DH5α	Cloning strain	Life Sciences
Top10	Cloning strain	Life Sciences
BL21 star (DE3)	Expression strain	Life Sciences
Rosetta 2	Expression strain	Novagen
N. gonorrhoeae		
MS11A	WT strain	1
SJ001	MS11 strain with *pilQ* truncation	2
SJ004-MS	*pilQ* with insertion encoding internal His-tag	This work
SJ082-MS	*ΔtsaP*	This work
KS035	*ΔtsaP/Plac-tsaP*	This work
SJ030-MS	MS11 strain transformed with pSJ030; nonpolar insertion in *pilC*, ErmC	2
SJ031-MS	MS11 strain transformed with PCR product to introduce in frame deletion of aa 1–181 of *pilP*	2
EP060	MS11 strain transformed with pEP057; nonpolar insertion in *pilF*, ErmC	2
SJ032-MS	MS11 strain transformed with pSJ032; nonpolar insertion of *pilW*, ErmC	2
M. xanthus		
DK1622	WT	3
DK8615	*ΔpilQ*	4
DK10409	*ΔpilT*	5
DK10410	*ΔpilA*	5
DK10416	*ΔpilB*	5
DK10417	*ΔpilC*	5
SA3001	*ΔpilO*	6
SA3002	*ΔpilM*	7
SA3005	*ΔpilP*	6
SA3044	*ΔpilN*	6
SA3045	*ΔpilT/yfp-pilT* (pIB75)	7
SA4070	*ΔpilP/*P_{pilA}*-pilP-sfGFP* (pSC102)	6
SA4088	*ΔpilO/*P_{pilA}*-pilO-sfGFP* (pSC106)	6
SA6011	*ΔtsaP*	This work
SA6012	WT/P_{pilA}*-tsaP-mCherry* (pIMB3)	This work
SA6013	*ΔtsaP/*P_{pilA}*-tsaP-mCherry* (pIMB3)	This work
SA6014	*ΔtsaP/*P_{pilA}*-tsaP* (pIMB2)	This work
SA6015	*ΔpilQ/*P_{pilA}*-tsaP-mCherry* (pIMB3)	This work
SA6022	*ΔpilP/*P_{pilA}*-tsaP-mCherry* (pIMB3)	This work
SA6026	WT/P_{pilA}*-tgl, pilQ-sfGFP* (pSC120)	6
SA6032	*ΔtsaP/*P_{pilA}*-tgl,pilQ-sfGFP* (pSC120)	This work
SA6041	*ΔtsaP/*P_{pilA}*-pilP-sfGFP* (pSC102)	This work
SA6043	*ΔpilA/*P_{pilA}*-tsaP-mCherry* (pIMB3)	This work
SA6044	*ΔpilB/*P_{pilA}*-tsaP-mCherry* (pIMB3)	This work
SA6045	*ΔpilC/*P_{pilA}*-tsaP-mCherry* (pIMB3)	This work
SA6046	*ΔpilM/*P_{pilA}*-tsaP-mCherry* (pIMB3)	This work
SA6047	*ΔpilO/*P_{pilA}*-tsaP-mCherry* (pIMB3)	This work
SA6048	*ΔpilT/*P_{pilA}*-tsaP-mCherry* (pIMB3)	This work
SA6049	*ΔpilN/*P_{pilA}*-tsaP-mCherry* (pIMB3)	This work
SA6050	*ΔtsaP/*P_{pilA}*-yfp-pilT* (pIB75)	This work
SA6051	*ΔtsaP/*P_{pilA}*-pilO-sfGFP* (pSC106)	This work
SA6053	*Δtgl*	6
SA6059	*Δtgl/*P_{pilA}*-tsaP-mCherry* (pIMB3)	This work

[a]In constructs with the P_{pilA}, genes were transcribed from the *pilA* promoter. All plasmids were integrated at the Mx8 *attB* site.

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