Supplemental Figures and Tables for:

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"Diverse C-terminal sequences involved in *Flavobacterium johnsoniae* protein secretion"

Fig. S1. Alignment of the C-terminal 100 amino acids of *F. johnsoniae* proteins that belong to TIGRFAM family TIGR04183 (type-A CTD). Protein sequences were aligned using MUSCLE. Dark shading indicates identical amino acids and light shading indicates similar amino acids. The red arrow indicates the conserved lysine that was mutated to alanine in RemA in the experiment shown in Fig. 6B of the main text. *F. johnsoniae* proteins that were examined experimentally in this study were RemA and Fjoh_1208 (AmyB). Proteins from other bacteria that were examined experimentally in this study were Celal_2532 (*C. algicola* AmyA), CHU_1335 (*C. hutchinsonii* Cel9B), and PGN_1466 (*P. gingivalis* RgpB).

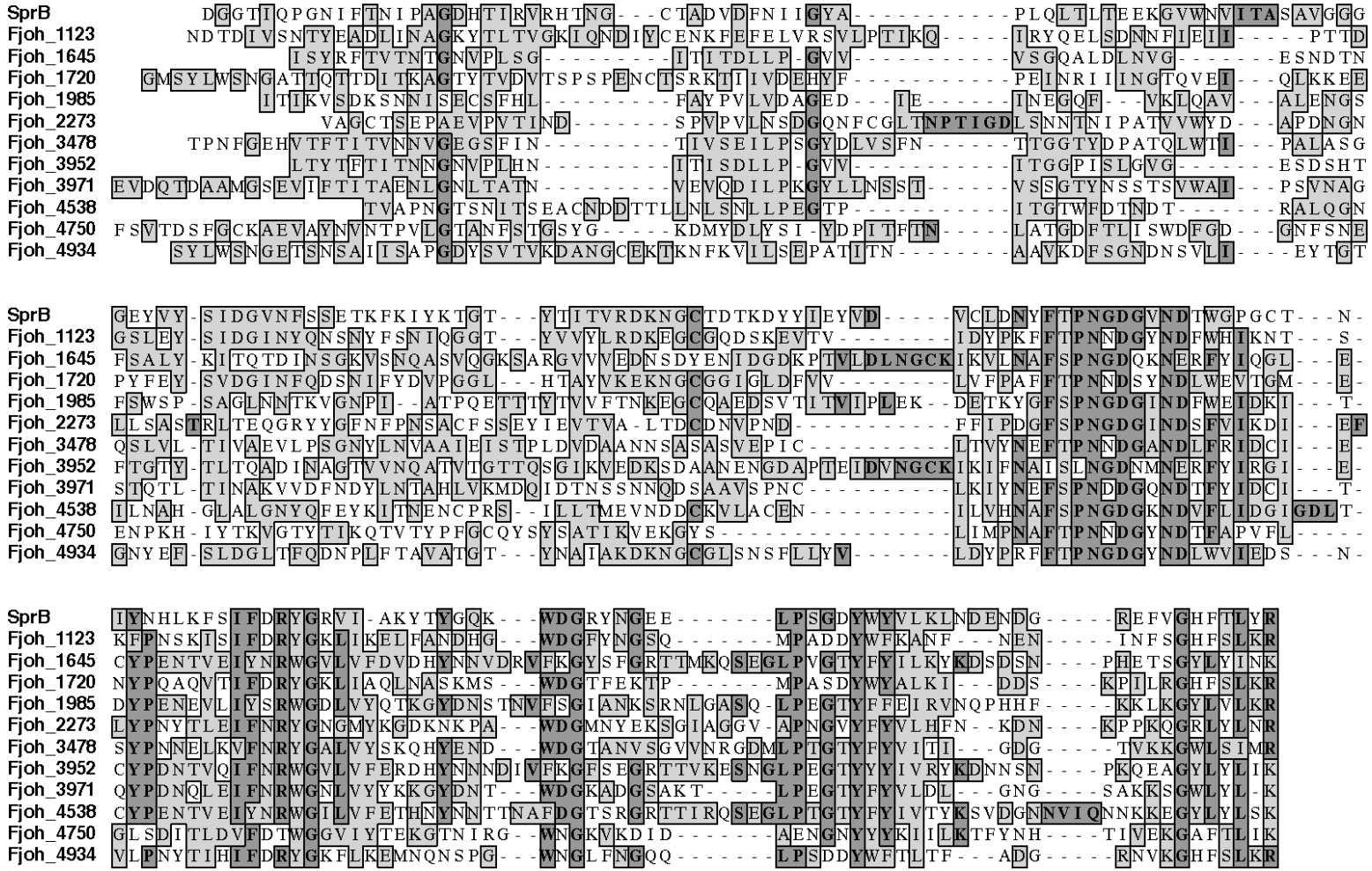


Fig. S2. Alignment of the C-terminal 200 amino acids of *F. johnsoniae* proteins that belong to TIGRFAM family TIGR04131 (type-B CTD). Protein sequences were aligned using MUSCLE. Dark shading indicates identical amino acids and light shading indicates similar amino acids. Proteins that were examined experimentally in this study were SprB and Fjoh_3952.

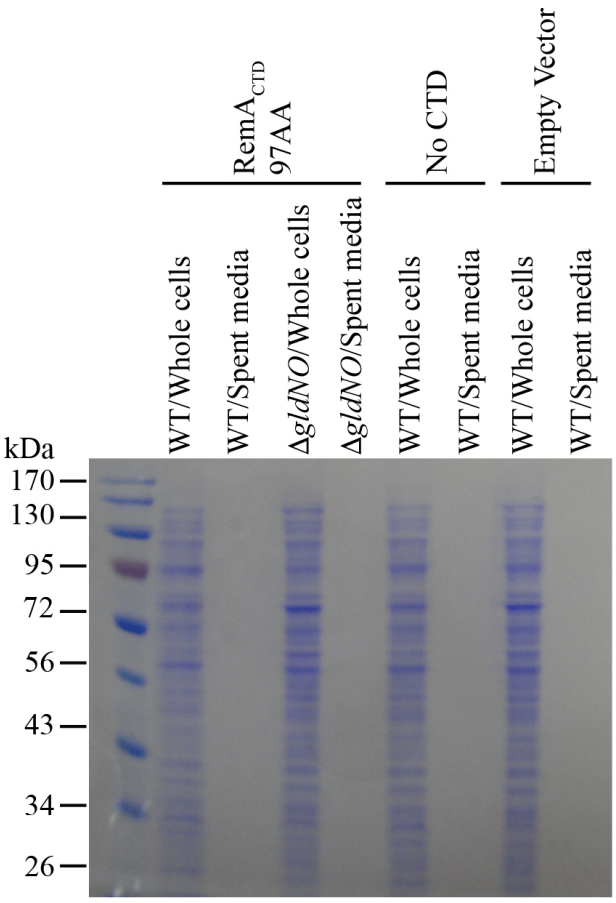


Fig. S3. Analysis of secreted proteins by SDS-PAGE. Cultures of wild type cells or of cells of the T9SS mutant Δ*gldNO* were incubated in CYE at 25°C with shaking and harvested in stationary phase (22 h). 1 ml samples were centrifuged at 22,000 x g for 15 min. The culture supernatant (spent medium) and intact cells were analyzed by SDS-PAGE followed by Coomassie blue staining. Cells carried either pCP23 ('Empty Vector'), pSK37 which expresses sfGFP with the N-terminal signal peptide from RemA (SP-sfGFP; 'No CTD'), or pSK30, which expresses SP-sfGFP fused to the 97-amino acid CTD of RemA (SP-sfGFP-CTD$_{RemA}$; 'RemA$_{CTD}$ 97 AA'). Cell samples corresponded to 10 µg protein per lane and samples from spent media corresponded to the volume of spent medium that contained 10 µg cell protein before the cells were removed.

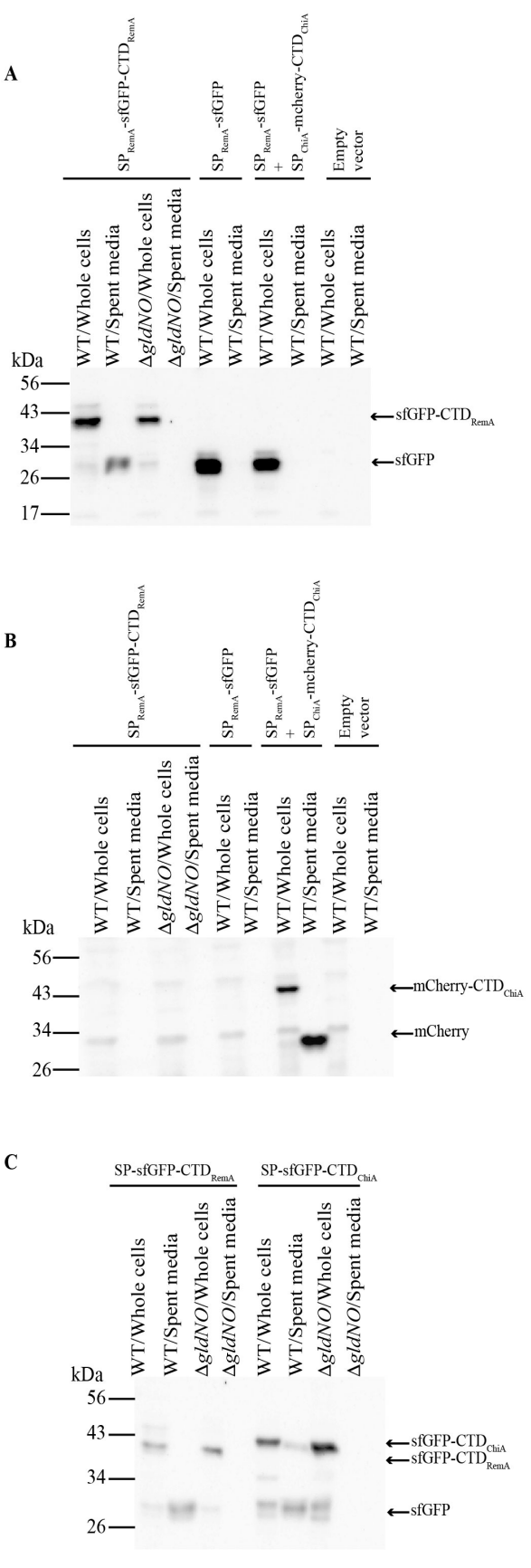


Fig. S4. Analysis of secreted proteins to determine if overexpression of CTD causes cell lysis or periplasmic leakage. Cultures of wild type cells or of the T9SS mutant Δ*gldNO* expressing SP_{RemA}-sfGFP-CTD_{RemA} (pSK30), SP_{RemA}-sfGFP (pSK37), or SP_{RemA}-sfGFP (pSK96) and SP_{ChiA}-mCherry-CTD_{ChiA} (pSSK52) were incubated in CYE at 25°C with shaking. 'Empty vector' refers to pCP23. 1 ml samples were centrifuged at 22,000 x g for 15 min. The culture supernatant (spent medium) and intact cells were analyzed by SDS-PAGE, followed by western blot analysis using (A) anti-GFP antibodies and (B) anti-mCherry antibodies. Identical samples were used in panels A and B. (C) To estimate protein expression from the *remA* and *chiA* promoters, cultures of wild type cells or of the T9SS mutant Δ*gldNO* expressing SP_{RemA}-sfGFP-CTD_{RemA} (pSK30; Pr_{remA}), or SP_{ChiA}-sfGFP-CTD_{ChiA} (pCB3; Pr_{chiA}) were incubated in CYE at 25°C with shaking. The culture supernatant (spent medium) and intact cells were analyzed by SDS-PAGE, followed by western blot analysis with anti-GFP antibodies. For all panels cell samples corresponded to 10 µg protein per lane and samples from spent media corresponded to the volume of spent medium that contained 10 µg cell protein before the cells were removed.

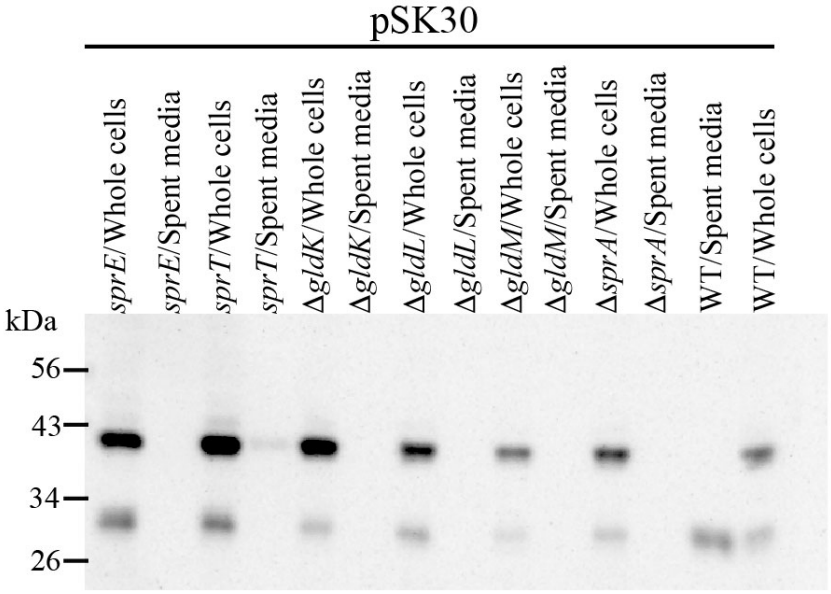


Fig. S5. Components of T9SS required for secretion of SP-sfGFP-CTD$_{RemA}$. Cultures of wild type cells (WT) or of the T9SS mutants were incubated in CYE at 25°C with shaking and harvested in stationary phase (22 h). 1 ml samples were centrifuged at 22,000 x g for 15 min. The culture supernatant (spent medium) and intact cells were analyzed for sfGFP by western blot. Cells carried pSK30, which expresses SP-sfGFP fused to the 97-amino acid CTD of RemA (SP-sfGFP-CTD$_{RemA}$). Whole cell samples corresponded to 10 μg protein per lane and samples from spent media corresponded to the volume of spent medium that contained 10 μg cell protein before the cells were removed. Samples were separated by SDS-PAGE, and sfGFP was detected using anti-serum against GFP.

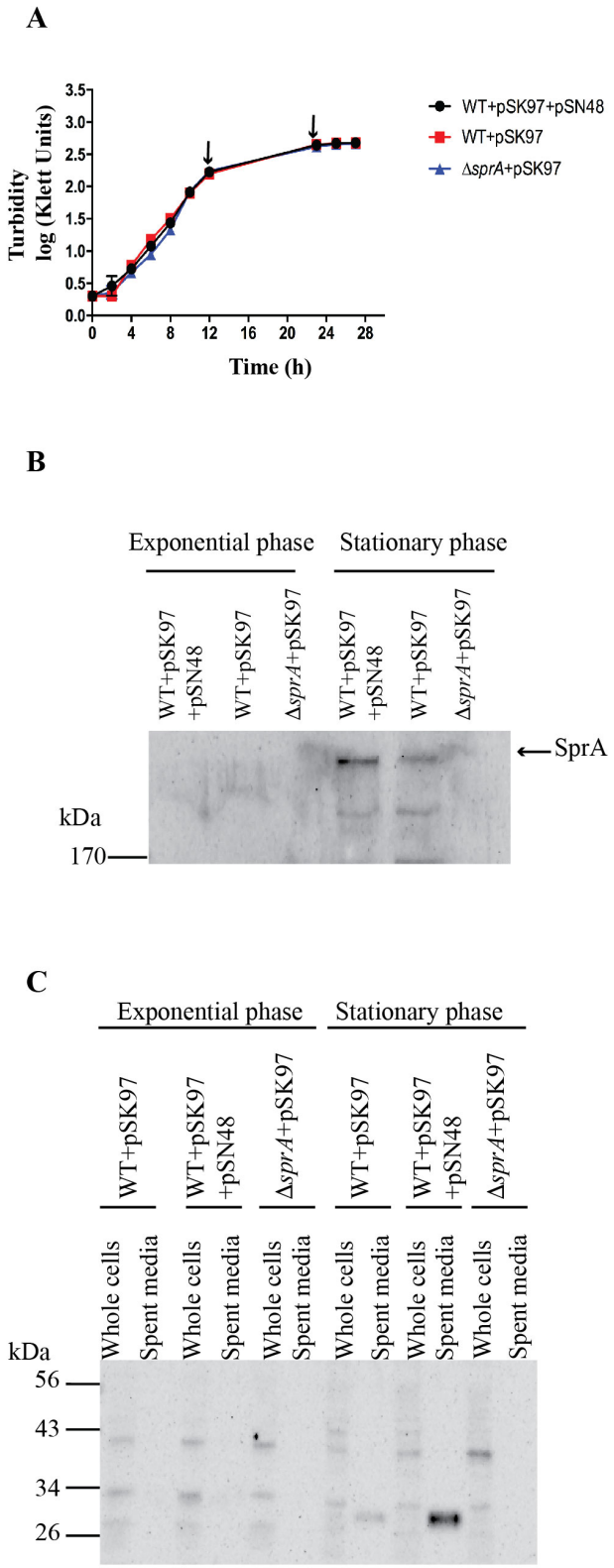


Fig. S6. Overexpression of SprA results in increased sfGFP secretion in stationary phase. Cultures of wild type cells or of cells of the T9SS mutant Δ*sprA* were incubated in CYE at 25°C with shaking and harvested in late exponential phase and stationary phase as indicated in Panel A. Cells carried pSK97 which expresses SP-sfGFP-CTD_{RemA} (97 AA CTD). Where indicated cells also carried pSN48 which expresses SprA. Cells were analyzed by western blot using anti-SprA antibodies (B) or anti-GFP antibodies (C). For Panel B, equal amounts (10 µg whole cell protein) were loaded per lane. For panel C, whole cell samples corresponded to 10 µg protein per lane and samples from spent media corresponded to the volume of spent medium that contained 10 µg cell protein before the cells were removed. Samples were separated by SDS-PAGE, and antibodies were used to detect the respective proteins.

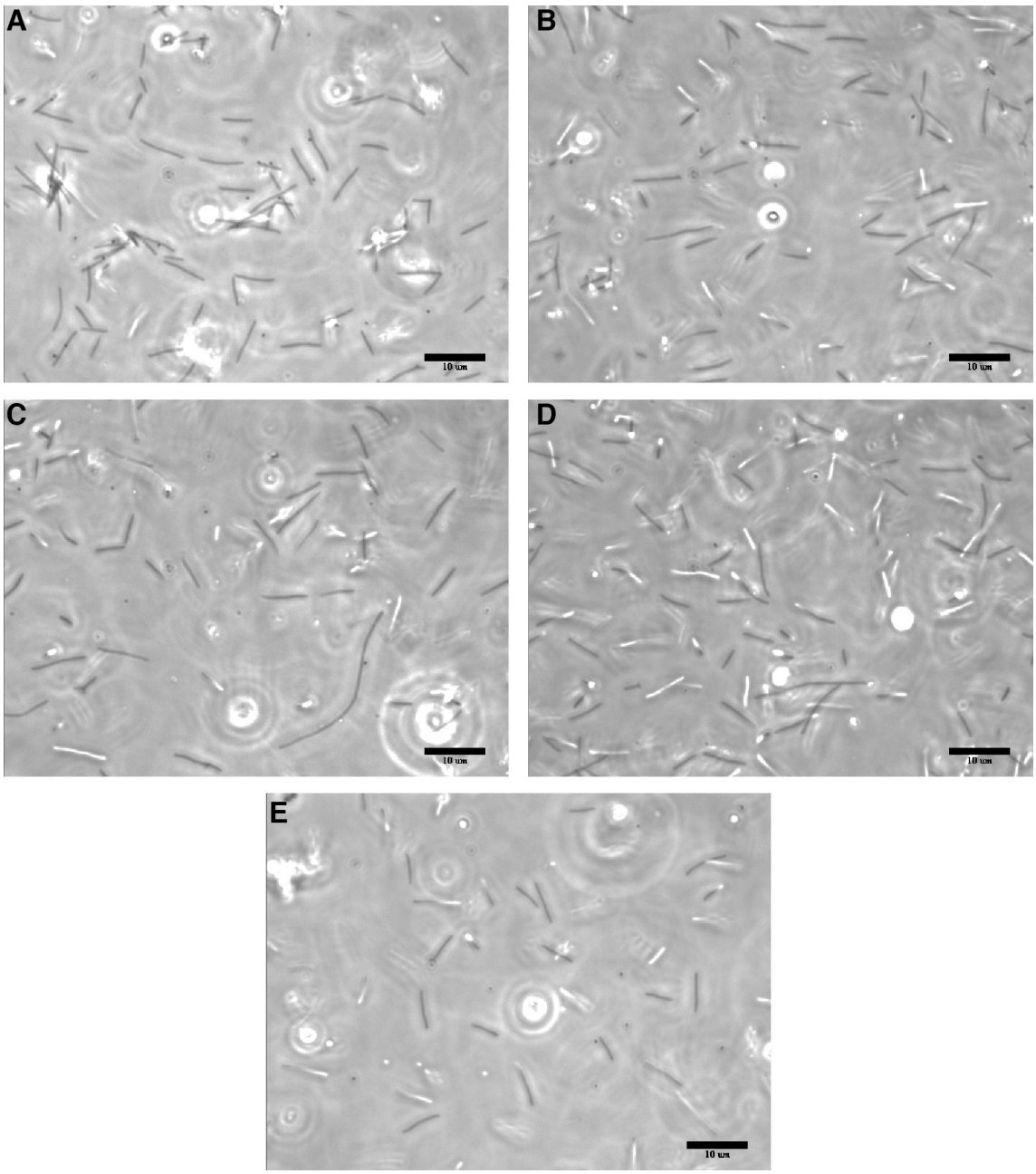
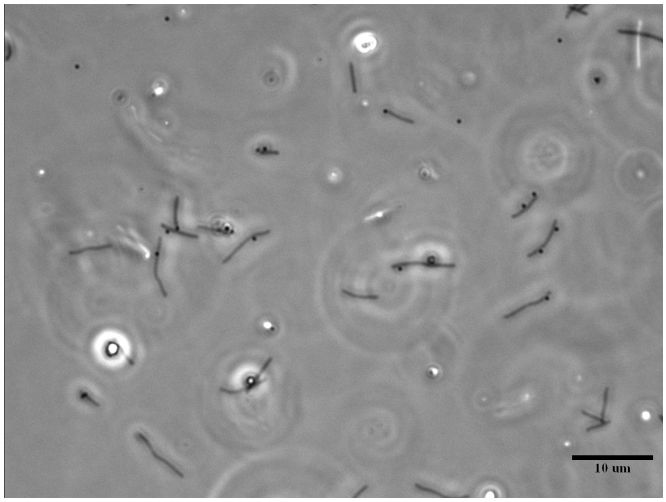


Fig. S7. Detection of cell-surface localized sfGFP. Protein G-coated 0.5-μm polystyrene spheres coated with anti-GFP antibodies were added to cells of *F. johnsoniae* and images were recorded using a Photometrics CoolSNAP$_{cf}^{2}$ camera mounted on an Olympus BH-2 phase-contrast microscope. Cells of wild-type *F. johnsoniae* expressing full length RemA with sfGFP fused after the signal peptide (A) attached to spheres, whereas cells expressing SP-sfGFP-CTD$_{RemA97AA}$ (B), SP-sfGFP-CTD$_{RemA87AA}$ (C), SP-sfGFP-CTD$_{RemA62AA}$ (D), and SP-sfGFP with no CTD (E) did not. Bars indicate 10 μm.

A



B

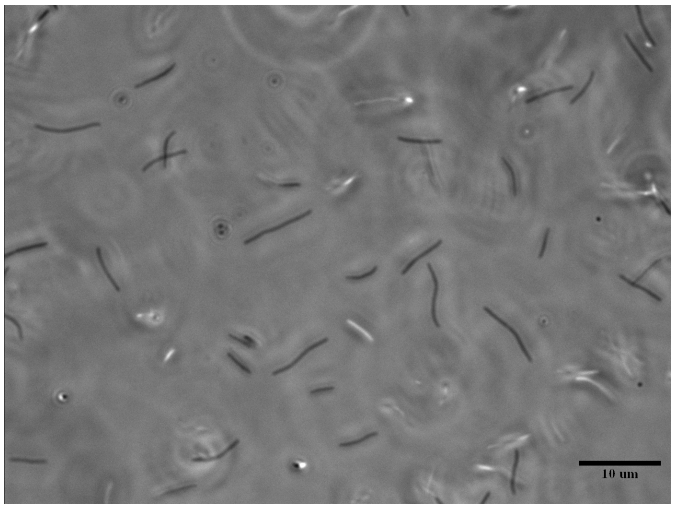


Fig. S8. Detection of cell-surface localized SprB. Protein G-coated 0.5-μm polystyrene spheres carrying anti-SprB antibodies were added to cells of *F. johnsoniae* and images were recorded using a Photometrics Cool-SNAP$_{cf}^2$ camera mounted on an Olympus BH-2 phase-contrast microscope. Cells of wild-type *F. johnsoniae* FJ1 attached to the spheres (A), whereas cells of the *sprB* transposon insertion mutant FJ117 which produces SprB lacking the C-terminal 34 amino acids (B) did not. Bars indicate 10 μm.

Table S1. Strains and plasmids used in this study.

Strain	Description[a]	Source or reference
***E. coli* strains**		
DH5αmcr	Strain used for general cloning	Life Technologies (Grand Island, NY, USA)
HB101	Strain used with pRK2013 for triparental conjugation	(1, 2)
***F. johnsoniae* strains**		
FJ1	wild type *F. johnsoniae* ATCC 17061^T	(3)
CJ1827	*rpsL2*; Sm^r 'wild-type' *F. johnsoniae* strain used in construction of deletion mutants	(4)
CJ2122	Δ*gldK*	(5)
CJ2157	Δ*gldL*	(5)
CJ2262	Δ*gldM*	(5)
CJ1631A	Δ(*gldN-gldO)*	(6)
CJ2302	Δ*sprA*	(5)
FJ149	*sprE*	(7)
CJ2518	Δ*sprF*	(8)
KDF002	*sprT*	(9)
CJ2116	Δ*porU*	(10)
CJ2130	Δ*porV*	(10)
CJ1922	Δ*sprB*	(4)
CJ1984	Δ*remA*	(11)
FJ117	*sprB HimarEm2* mutant	(12)
FJ156	*sprB HimarEm2* mutant	(12)

Plasmid	Description	Source or reference
pCB3	735-bp sfGFP without stop codon amplified and cloned into pSSK52. Encodes SP_{ChiA}-sfGFP-$CTD_{ChiA(105AA)}$; Ap^r (Tc^r)	This study
pCB4	440-bp region encoding 62 amino acids of CTD_{ChiA} inserted into pCB3. Encodes SP_{ChiA}-sfGFP-$CTD_{ChiA(62AA)}$; Ap^r (Tc^r)	This study
pCP11	*E. coli-F. johnsoniae* shuttle plasmid; Ap^r (Em^r)	(13)
pCP23	*E. coli-F. johnsoniae* shuttle plasmid; Ap^r (Tc^r)	(14)
pMM105.A	*E. coli-Capnocytophaga canimorsus* shuttle plasmid; Ap^r (Em^r)	(15)
pRK2013	Helper plasmid for triparental conjugation; IncP Tra$^+$ Km^r	(2)
pRR48	1294-bp fragment spanning *sprF* inserted into pCP23;	(16)

	Ap^r (Tc^r)	
pSK30	339-bp region encoding 97 amino acids of CTD_{RemA} inserted into pYT179. Encodes SP_{RemA}-sfGFP-$CTD_{RemA(97AA)}$; Ap^r (Tc^r)	This study
pSK37	SP_{RemA}-sfGFP with stop codon cloned into pYT40. Encodes SP-sfGFP; Ap^r (Tc^r)	This study
pSK56	657-bp region encoding 218 amino acids of CTD_{SprB} inserted into pYT179. Encodes SP-sfGFP- $CTD_{SprB(218AA)}$; Ap^r (Tc^r)	This study
pSK58	687-bp region encoding 228 amino acids of CTD_{Fjoh_3952} inserted into pYT179. Encodes SP-sfGFP-$CTD_{Fjoh\_3952(228AA)}$; Ap^r (Tc^r)	This study
pSK62	3549-bp region encoding 1182 amino acids of CTD_{SprB} inserted into pYT179. Encodes SP-sfGFP-$CTD_{SprB(1182AA)}$; Ap^r (Tc^r)	This study
pSK65	417-bp region encoding 108 amino acids of CTD_{Celal_2532} inserted into pYT179. Encodes SP-sfGFP- CTD_{Celal_2532}; Ap^r (Tc^r)	This study
pSK71	312-bp region encoding 87 amino acids of CTD_{RemA} inserted into pYT179. Encodes SP-sfGFP- $CTD_{RemA(87AA)}$; Ap^r (Tc^r)	This study
pSK75	339-bp region encoding 103 amino acids of CTD_{PGN_1466} inserted into pYT179. Encodes SP-sfGFP- CTD_{PGN_1466}; Ap^r (Tc^r)	This study
pSK76	294-bp region encoding 97 amino acids of CTD_{CHU_1335} inserted into pYT179 Encodes SP-sfGFP- CTD_{CHU_1335}; Ap^r (Tc^r)	This study
pSK79	258-bp region encoding 85 amino acids near the C-terminus of RemA but lacking the C-terminal 12 amino acids inserted into pYT179. Encodes SP-sfGFP-$CTD_{RemA(lacking\ final\ 12\ AA)}$; Ap^r (Tc^r)	This study
pSK81	234-bp region encoding 62 amino acids of CTD_{RemA} inserted into pYT179. Encodes SP-sfGFP- $CTD_{RemA(62AA)}$; Ap^r (Tc^r)	This study
pSK82	390-bp region encoding 99 amino acids of CTD_{AmyB} inserted into pYT179. Encodes SP-sfGFP- $CTD_{AmyB(99AA)}$; Ap^r (Tc^r)	This study
pSK84	396-bp fragment spanning the Fjoh_1634 promoter, start codon, and the N-terminal signal peptide-encoding region inserted into pSK30. Encodes SP_{Fjoh_1634}-sfGFP-CTD_{RemA}; Ap^r (Tc^r)	This study
pSK85	312-bp region encoding 73 amino acids of CTD_{AmyB} inserted into pYT179. Encodes SP-sfGFP- $CTD_{AmyB(73AA)}$; Ap^r (Tc^r)	This study
pSK86	270-bp region encoding 59 amino acids of CTD_{AmyB} inserted into pYT179. Encodes SP-sfGFP- $CTD_{AmyB(59AA)}$;	This study

	Ap^r (Tc^r)	
pSK89	491-bp region encoding 79 amino acids of CTD_{ChiA} inserted into pCB3. Encodes SP_{ChiA}-sfGFP-$CTD_{ChiA(79AA)}$; Ap^r (Tc^r)	This study
pSK91	300-bp region encoding CTD_{RemA} with K1432A mutation inserted into pYT179. Encodes SP-sfGFP-$CTD_{RemA(K1432A)}$; Ap^r (Tc^r)	This study
pSK93	300-bp region encoding 99 amino acids of CTD_{SprB} inserted into pYT179. Encodes SP-sfGFP- $CTD_{SprB(99AA)}$; Ap^r (Tc^r)	This study
pSK96	SP_{RemA}-sfGFP from pSK37 cloned into pCP11. Encodes SP_{RemA}-sfGFP; Ap^r (Em^r)	This study
pSK97	SP_{RemA}-sfGFP-CTD_{RemA} from pSK30 inserted into pMM105.A. Encodes SP_{RemA}-sfGFP-$CTD_{RemA(97AA)}$; Ap^r (Em^r)	This study
pSN48	pCP23 carrying *sprA*; Ap^r (Tc^r)	(17)
pSSK30	pCP23 carrying mcherry; Ap^r (Tc^r)	(18)
pSSK51	484-bp fragment spanning the *chiA* promoter, start codon, and N-terminal signal peptide-encoding region inserted into pSSK30. Encodes SP_{ChiA}-mCherry; Ap^r (Tc^r)	(18)
pSSK52	566-bp region encoding 105 amino acids of CTD_{ChiA} inserted into pSSK51. Encodes SP_{ChiA}-mCherry-CTD_{ChiA}; Ap^r (Tc^r)	(18)
pTB263	Plasmid expressing fluorescent protein sfGFP; Ap^r	(19)
pYT40	511-bp fragment spanning the *remA* promoter, start codon, and the N-terminal signal peptide-encoding region inserted into pCP23; Ap^r (Tc^r)	This study
pYT179	735-bp sfGFP amplified without stop codon and cloned into pYT40. Encodes SP_{RemA}-sfGFP; Ap^r (Tc^r)	This study
pYT180	4383-bp fragment encoding 1386 amino acids of the C-terminus of RemA inserted in pYT179. Encodes SP_{RemA}-sfGFP-$CTD_{RemA(1386AA)}$; Ap^r (Tc^r)	This study

[a]Antibiotic resistance phenotypes are as follows: ampicillin, Ap^r; erythromycin, Em^r; streptomycin, Sm^r; tetracycline, Tc^r. The antibiotic resistance phenotypes given in parentheses are those expressed in *F. johnsoniae* but not in *E. coli*. The antibiotic resistance phenotypes without parentheses are those expressed in *E. coli* but not in *F. johnsoniae*.

Table S2. Primers used in this study

1269	5' GCTAGGGTACCACGTTCCTGATAGGCACAAAAATGC 3'; forward primer used in construction of pYT40; KpnI site underlined
1270	5' GCTAGGGATCCGCCATTAGTTGGCATTCCAGGAAAA 3'; reverse primer used in construction of pYT40; BamHI site underlined
1389	5' GCTAGGGATCCTCTAAAGGTGAAGAACTGTTCACCG 3'; forward primer used in construction of pSK37 and pYT179; BamHI site underlined
1390	5' GCTAGGCATGCTTATTTGTAGAGCTCATCCATGCCG 3'; reverse primer used in construction of pSK37; SphI site underlined
1399	5’ GCTAGTCTAGAACAGATACGAAAGATTATTACATCGAG 3'; forward primer used in construction of pSK93; XbaI site underlined
1400	5' GCTAGGCATGCTTATCTGTATAAAGTGAAATGTCCAAC 3'; reverse primer used in construction of pSK56; SphI site underlined
1404	5' GCTAGGCATGCTCACCTAATACAATAACTAACCTC 3'; reverse primer used in construction of pSSK52; SphI site underlined
1427	5’ GCTAGTCTAGAGCAACGATAGCTTATTTTAAAAACAAT 3’; forward primer used in construction of pSK89; XbaI site is underlined
1488	5' GCTAGTCTAGAGATCGTTTTGCACTTCGTTACACT 3'; forward primer used in construction of pSK30; XbaI site underlined
1489	5' GCTAGGCATGCCTTACTTGGCAAATGGATTTTTTA 3'; reverse primer used in construction of pSK30; SphI site underlined
1599	5' GCTAGTCTAGAGCAACGATAGCTTATTTTAAAAACAAT 3'; forward primer used in construction of pSK89; XbaI site underlined
1600	5' GCTAGTCTAGAGCTTATGCAGCTTATTTCGCATCACAA 3'; forward primer used in construction of pSSK52; XbaI site underlined
1771	5' GCTAGGGATCCCTAACCCGACTATCATAGAACCGAC 3'; forward primer used in construction of pYT314; BamHI site underlined
1772	5' GCTAGGTCGACTGTTGTTACAGCCATGAGTACTAAGG 3'; reverse primer used in construction of pYT314; SalI site underlined
1773	5' GCTAGGTCGACTCGATTAGTAACTGTCCTTGTACGC 3'; forward primer used in construction of pYT316; SalI site underlined
1774	5' GCTAGGCATGCTAAAAGTTCAGTTGGCAGTTCTTCG 3'; reverse primer used in construction of pYT316; SphI site underlined
1880	5’ GCTAGGCATGCTGGCGAGGAATTACCTTCTGGTGA 3'; forward primer used in construction of pSK62; XbaI site underlined
1843	5’ GCTAGTCTAGAGTGGTGATTACAATTGATCCAAGC 3'; forward primer used in construction of pSK56; XbaI site underlined
1868	5’ GCTAGTCTAGAGTCGAAGTGCCATCGATTACAGTA 3'; forward primer used in construction of pSK58; XbaI site underlined
1885	5' GCTAGTCTAGAGCTTTAGAGGCTTTTGAAAATGTG 3'; forward primer used in construction of pSK65; XbaI site underlined
1886	5' GCTAGGCATGCTTGTGGGCGTTTCTGAACTATCTC 3'; reverse primer used in construction of pSK65; SphI site underlined

1870 5' GCTAGGCATGCGCTAAGCCATTTTATTGATTTGGA 3'; reverse primer used in construction of pSK58; SphI site underlined

1899 5' GCTAGTCTAGAACATTAGGAACTGGTGATTTTGAG 3'; forward primer used in construction of pSK71; XbaI site underlined

1923 5' GCTAGTCTAGAGAGAGTATCGCTGATGAAACGAAC 3'; forward primer used in construction of pSK75; XbaI site underlined

1924 5' GCTAG GCATGC GCCCTTATTAGAGAATTGCAGTGT 3'; reverse primer used in construction of pSK75; SphI site underlined

1925 5' GCTAGTCTAGAGTATCGGTAAGTGTGGGAACTCCT 3'; forward primer used in construction of pSK76; XbaI site underlined

1926 5' GCTAGGCATGCCTGTATAGGCTATTCTTTTATAAGGCG 3'; reverse primer used in construction of pSK76; SphI site underlined

1930 5' GCTAGTCTAGAACTTCTGCAAAAGAAAATATTAAAGAA 3'; forward primer used in construction of pSK81; XbaI site underlined

1932 5' GCTAGGCATGCCTATTCAAGATTAACTTTTACAAGCAGCAC 3'; reverse primer used in construction of pSK79; SphI site underlined

1933 5' GCTAGTCTAGAGAACCAACAACTGTTGGAACAGGA 3'; forward primer used in construction of pSK82; XbaI site underlined

1934 5' GCTAGGCATGCCGAATCGAACAATAGCGAACAAGC 3'; reverse primer used in construction of pSK82; SphI site underlined

1940 5' GCTAGTCTAGAGAAGACATTGCTCAGGTTGATGTA 3'; forward primer used in construction of pCB4; XbaI site underlined

1946 5' GCTAGGGTACCGCTTTGAGCATGAATATTGTATCC 3'; forward primer used in construction of pSK84; KpnI site underlined

1947 5' GCTAGGGATCCATCTTGAGCAAATGAAGTTAGGGA 3'; reverse primer used in construction of pSK84; BamHI site underlined

1948 5' GCTAGTCTAGATATCCAAACCCATCTGTAAACAATGAA 3'; forward primer used in construction of pSK85; XbaI site underlined

1949 5' GCTAGTCTAGACCAGAATTGGAAAGCGGAGAC 3'; forward primer used in construction of pSK86; XbaI site underlined

1962 5' GCTAGGCATGCCTATTTAAAGATCACTGCTCTGGTTATCTG 3'; reverse primer used in construction of pSK91; SphI site underlined

Table S3. Prevalence of T9SS genes and CTD-encoding genes in 104 members of the phylum *Bacteroidetes*[a].

	T9SS components					CTDs	
	GldK	GldL	GldM	GldN	SprA	Type A CTD	Type B CTD
Genome	TIGR03525	TIGR03513	TIGR03517	TIGR03523	TIGR04189	TIGR04183	TIGR04131
Class Flavobacteriia							
Aequorivita sublithincola DSM 14238	1	1	1	1	1	112	5
Algibacter sp. HZ22	1	1	1	1	1	55	10
Capnocytophaga canimorsus Cc5	1	1	1	1	1	1	10
Capnocytophaga haemolytica CCUG 32990	1	1	1	1	1	2	6
Capnocytophaga ochracea DSM 7271	1	1	1	1	1	2	8
Capnocytophaga sp. F0383	1	1	1	1	1	2	7
Cellulophaga algicola DSM 14237	1	1	1	1	1	13	16
Cellulophaga lytica DSM 7489	1	1	1	1	1	14	13
Chryseobacterium sp. IHB B 17019	1	1	1	1	1	83	18
Chryseobacterium sp. StRB126	1	1	1	1	1	101	10
Croceibacter atlanticus HTCC2559	1	1	1	1	1	45	8
Dokdonia sp. PRO95	1	1	1	1	1	17	10
Donghaeana dokdonensis DSW-6	1	1	1	1	1	85	17
Elizabethkingia meningoseptica FMS-007	0	0	0	0	0	0	0
Elizabethkingia sp. BM10	0	0	0	0	0	0	0
Flavobacteriaceae bacterium 3519-10	1	1	1	1	1	55	6
Flavobacterium branchiophilum FL-15	1	1	1	1	1	37	10
Flavobacterium columnare ATCC 49512	1	1	1	1	1	35	7
Flavobacterium indicum GPTSA100-9	1	1	1	1	1	43	16
Flavobacterium johnsoniae ATCC 17061	1	1	1	2	1	40	12
Flavobacterium psychrophilum JIP02/86	1	1	1	1	1	38	10
Fluviicola taffensis DSM 16823	1	1	2	2	1	180	50
Gramella forsetii KT0803	1	1	1	1	1	11	7
Krokinobacter diaphorus 4H-3-7-5	1	1	1	1	1	15	10
Lacinutrix sp. 5H-3-7-4	1	1	1	1	1	31	16
Lutibacter profundi LP1	1	1	1	1	1	19	3
Maribacter sp. HTCC2170	1	1	1	1	1	10	13
Muricauda lutaonensis CC-HSB-11	1	1	1	1	1	7	13
Muricauda ruestringensis DSM 13258	1	1	1	1	1	7	13
Myroides profundi D25	1	1	1	1	1	7	12
Myroides sp. A21	1	1	1	1	1	4	7
Ornithobacterium rhinotracheale DSM 15997	1	1	1	1	1	6	2
Owenweeksia hongkongensis DSM 17368	1	1	1	1	1	159	26
Polaribacter sp. MED152	1	1	1	1	1	27	8
Riemerella anatipestifer DSM 15868	1	1	1	1	1	15	1
Robiginitalea biformata HTCC2501	1	1	1	1	1	7	12
Siansivirga zeaxanthinifaciens CC-SAMT-1	1	1	1	1	1	51	10
Weeksella virosa DSM 16922	1	1	1	1	1	36	3
Winogradskyella sp. PG-2	1	1	1	1	1	66	17
Zobellia galactanivorans DsijT	1	1	1	1	1	29	17
Zunongwangia profunda SM-A87	1	1	1	1	1	7	5
Class Cytophagia							
Belliella baltica DSM 15883	1	1	1	1	1	11	4
Cyclobacterium amurskyense KCTC 12363	1	1	1	1	1	20	5
Cyclobacterium marinum DSM 745	1	1	1	1	1	18	7
Cytophaga hutchinsonii ATCC 33406	1	1	2	2	1	118	27
Dyadobacter fermentans DSM 18053	1	1	1	1	1	88	11
Echinicola vietnamensis DSM 17526	1	1	1	1	1	17	9
Emticicia oligotrophica DSM 17448	1	1	1	1	1	31	10
Flexibacter litoralis DSM 6794	1	1	1	3	1	52	11
Hymenobacter sp. APR13	1	1	1	1	1	83	9
Hymenobacter sp. DG25A	1	1	1	1	1	54	6
Hymenobacter sp. PAMC26554	1	1	1	1	1	51	8
Hymenobacter swuensis DY53	1	1	1	1	1	100	8
Leadbetterella byssophila DSM 17132	1	1	1	1	1	17	4
Marivirga tractuosa DSM 4126	1	1	1	1	1	39	11
Persicobacter sp. JZB09	1	1	1	1	1	32	3
Pontibacter akesuensis AKS 1T	1	1	1	1	1	47	11
Pontibacter korlensis X14-1T	1	1	1	1	1	47	11
Rufibacter sp. DG15C	1	1	1	1	1	49	13
Rufibacter tibetensis 1351	1	1	1	1	1	56	11

Runella slithyformis DSM 19594	1	1	1	1	1	33	18
Spirosoma linguale DSM 74	1	1	1	1	1	53	14
Spirosoma radiotolerans DG5A	1	1	1	1	1	50	15
Class Sphingobacteriia							
Algoriphagus sp. M8-2	1	1	1	1	1	16	6
Arachidicoccus sp. BS20	2	1	1	2	1	11	0
Chitinophaga pinensis DSM 2588	1	1	1	1	1	51	36
Haliscomenobacter hydrossis DSM 1100	1	1	1	1	1	144	36
Mucilaginibacter PAMC26640	1	1	1	2	1	7	9
Niabella soli DSM 19437	0	0	0	0	0	0	0
Niastella koreensis DSM 17620	1	1	1	1	1	111	31
Pedobacter cryoconitis PAMC 27485	1	1	1	1	1	3	5
Pedobacter heparinus DSM 2366	1	1	1	1	1	8	13
Pedobacter saltans DSM 12145	1	1	1	1	1	29	10
Pedobacter sp. PACM 27299	1	1	1	1	1	1	9
Saprospira grandis Lewin	1	1	1	3	2	67	16
Solitalea canadensis DSM 3403	1	1	1	1	1	6	18
Sphingobacterium sp. 21	2	1	1	2	1	1	2
Sphingobacterium sp. ML3W	1	1	1	1	0	1	0
Class Bacteroidia							
Alistipes finegoldii DSM 17242	0	0	0	0	0	0	0
Bacteroides cellulosilyticus WH2	1	1	1	1	1	19	1
Bacteroides dorei CL03T12C01	0	0	0	0	0	0	0
Bacteroides fragilis NCTC 9343	0	1	1	0	0	0	0
Bacteroides helcogenes DSM 20613	0	0	0	0	0	0	0
Bacteroides ovatus ATCC 8483	0	0	0	0	0	0	0
Bacteroides thetaiotaomicron VPI-5482	0	0	0	0	0	0	0
Bacteroides vulgatus ATCC 8482	0	0	0	0	0	0	0
Bacteroides xylanisolvens XB1A	0	0	0	0	0	0	0
Barnesiella viscericola DSM 18177	1	1	1	1	1	41	1
Draconibacterium orientale FH5	2	1	1	1	1	23	7
Odoribacter splanchnicus DSM 20712	0	0	0	0	2	2	1
Paludibacter propionicigenes DSM 17365	1	1	1	1	1	10	6
Parabacteroides distasonis ATCC 8503	1	1	1	1	1	7	1
Porphyromonas asaccharolytica DSM 20707	1	1	1	1	1	29	1
Porphyromonas gingivalis ATCC 33277	1	1	1	1	1	17	1
Prevotella dentalis EDSM 3688	1	1	1	1	1	9	1
Prevotella denticola F0289	1	1	1	1	1	8	1
Prevotella enoeca F0113	1	1	1	1	1	4	1
Prevotella fusca W1435	1	1	1	1	1	6	1
Prevotella intermedia 17-2	1	1	1	1	1	19	1
Prevotella melaninogenica ATCC 25845	1	1	1	1	1	14	1
Prevotella ruminicola 23	1	1	1	1	1	1	1
Prevotella sp. F0039	1	1	1	1	1	13	1
Rikenellaceae bacterium M3	0	0	0	0	0	0	0
Tannerella forsythia ATCC 43037	1	1	1	1	1	28	0
	T9SS components					CTDs	
	GldK	GldL	GldM	GldN	SprA	Type A CTD	Type B CTD
	TIGR03525	TIGR03513	TIGR03517	TIGR03523	TIGR04189	TIGR04183	TIGR04131

[a]Only members of the *Bacteroidetes* with completed genome sequences were examined and only one member of each species was used. Occurrence of genes encoding T9SS components or of genes encoding proteins with T9SS-associated CTDs are shown. Red indicates the presence of a gene and the number indicates the number of such genes in the genome. Genes were identified using the Integrated Microbial Genomes (IMG version 4.0.1) Function Profile Tool and using the TIGRFAM terms listed. The trusted cutoffs set by The Institute for Genomic Research were used in each case as indicated in the Methods section of the main text. These may underrepresent the actual number of proteins secreted by T9SSs. For example, more than 30 proteins are thought to be secreted by the *P. gingivalis* T9SS (20), but only 18 were identified above.

Table S4. Prevalence of T9SS genes and CTD-encoding genes in organisms outside of the phylum *Bacteroidetes*[a].

	T9SS components					CTDs	
	GldK	GldL	GldM	GldN	SprA	Type A CTD	Type B CTD
Genome	TIGR03525	TIGR03513	TIGR03517	TIGR03523	TIGR04189	TIGR04183	TIGR04131
Bacteria (non *Bacteroidetes* , 3777 genomes examined)							
Chloroherpeton thallassium ATCC 35110 (Chlorobi)	0	0	0	0	1	30	0
Arthrospira platensis YZ (Cyanobacteria)	0	0	0	0	0	1	0
Leptolyngbya sp. PCC 7376 (Cyanobacteria)	0	0	0	0	0	1	0
Synechococcus sp. JA-2-3B'a(2-13) (Cyanobacteria)	0	0	0	0	0	1	0
Trichodesmium erythraeum IMS101 (Cyanobacteria)	0	0	0	0	0	1	0
Fibrobacter succinogenes S85 (Fibrobacteres)	0	0	0	0	1	33	0
Thermincola potens JR (Firmicutes)	0	0	0	0	0	1	0
Gemmatimonas aurantiaca T-27T (Gemmatimonadetes)	0	0	0	0	1	0	0
Melioribacter roseus P3M (Ignavibacteriae)	0	0	0	0	1	85	0
Ignavibacterium album JCM 16511 (Ignavibacteriae)	0	0	0	0	1	147	0
Rhodothermus marinus DSM 4252 (Rhodothermaeota)	0	0	0	0	2	48	0
Salinibacter ruber DSM 13855 (Rhodothermaeota)	0	0	0	0	1	18	0
Archaea (218 genomes examined)							
No species identified with T9SS genes or CTDs	0	0	0	0	0	0	0
Eukarya (36 genomes examined)							
No species identified with T9SS genes or CTDs	0	0	0	0	0	0	0

[a]3777 completed genomes were examined. Only completed genome sequences were examined and only one member of each species was used. Since the vast majority of species had no genes encoding T9SS proteins or T9SS-associated CTDs, only species with genes encoding T9SS components or genes encoding proteins with T9SS-associated CTDs are shown. Red indicates the presence of a gene and the number indicates the number of such genes in the genome. Genes were identified using the Integrated Microbial Genomes (IMG version 4.0.1) Function Profile Tool and using the TIGRFAM terms listed. The trusted cutoffs set by The Institute for Genomic Research were used in each case as indicated in the Methods section of the main text. The phyla to which the species belong are indicated in parentheses. Note that the CTDs from *A. platensis*, *Leptolyngbya* sp., and *T. erythraeum* were not found at the C-terminus and thus may be false positives.

Table S5. Amino acid sequence identities of *F. johnsoniae* T9SS components with orthologs from other members of the phylum *Bacteroidetes*[a].

F. johnsoniae	*C. algicola*	*C. hutchinsonii*	*P. gingivalis*
GldK	67% over 467 AA	33% over 477 AA	34% over 502 AA
GldL	58% over 219 AA	27% over 273 AA	19% over 313 AA
GldM	41% over 526 AA	20% over 546 AA	24% over 539 AA
GldN	52% over 334 AA	14% over 353 AA	16% over 409 AA
SprA	52% over 2460 AA	32% over 2537 AA	32% over 2622 AA
SprE	40% over 887 AA	21% over 902 AA	15% over 1191 AA
SprT	48% over 240 AA	25% over 244 AA	22% over 254 AA
PorU	No ortholog	33% over 1332 AA	23% over 1335 AA
PorV	57% over 404 AA	34% over 417 AA	42% over 413 AA

[a]*F. johnsoniae* T9SS components were aligned with orthologs from *Cellulophaga algicola* (Class *Flavobacteriia*), *Cytophaga hutchinsonii* (Class *Cytophagia*) and *Porphyromonas gingivalis* (Class *Bacteroidia*) using MUSCLE. In each case percent amino acid (AA) identity over the region of similarity is listed. Note that *C. algicola*, which has a functional T9SS, lacks a PorU ortholog.

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