Supplemental Figures and Tables for:

Surashree S. Kulkarni, Yongtao Zhu, Colton J. Brendel and Mark J. McBride.

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

				•
Fjoh 0074	LKODVLSTP-DEGTKNGEVVKAVPNPT-S-DVIN	TVKT NESKNLKLRLYDLN-	GRALGNPIDIOSSE - EVNTTV-MS	LRNLNAGLYIYTL SENNKVV YKNKII LKN
Fjoh_0547	AFFFTVSVSAQDSKQLPKT-QDPTGIEG-LSLYPNPVVN-GKVYI	ISSKN DLEKEIIVFDIL -	GKKVLQ AHLT . TKELNV	- SDLVPGVYIIRISEQN - AT ATRKLIIR
Fjoh_0549	GNRNILMSVDDSK TNTESNE - NNNITVIPVTFVNPFAK - T - VQSNV	/NVED SIQPYSINVYN FE -	GQKVLTKEV-KSIE-EENKSL	- DSLKSGIYIIKSKNETRKVLK
Fjoh_0707	SFKGIA-TGANEDLNNVYVYPNPV-R-PTYSC	JTVKVAGLIDKANIKITDIE-	GNLVYETTS-DGGT-IEWDTTAFG	RYKVSSGVYMIFISAQD-GSETKVKKVMIIR
Fjoh_0798	SASVNICTOVFTL - DTIDVVKKDFVLYPNPN - K - GSFTV	/QFKSESTSVKVFVNDIL-	GKTIYAKTF - ETDG - DFNQN I FL-	- PNAASGLYLVTVIDGD - KR TVRKIIIN
Fjon_0848	LAYDEFDLDALLEDACNKR-QPEKEVKIKPYVFPNPA-Q-TTITV	<u>/ENLN</u> SKNFDFEFFNFE-	SKSVLKGKTS-DGTINI	- LGLDSGFYILKTTIGE - TV ETFKVIKE
Fjon_UB86	E DYTTINI TAISGTTEIAEPTIA A - GTVETETKG FALYPNPU- E - ISELN	TVSEENAYSYKIINAL-		- SRLSTGIYLTELNNGK - EK IVKKFAKK
Fj011_1022	SFLLIGNFYLFLNANLSNDIFDIERSIVILTPNPS-F-DRIIN		COLVERTINGNUT DUTING	
Figh 1189	NWSNIKESTTTVSNTCKTL, GVPESALKNVVIH PNP T, K. GELHI		COLVESTINSNNT, DUTINI.	SGI PKGVYVVVI INOD. AA SAKKVIVE
Fioh 1208		VVLPE DESCIDMAS I SV SDIN-	GR TVI TE RISS SGRUD	- HRLASGI VI VNI VSKE - YK TTKKI I VK
Fioh 1231	NLFYMVTAYNTATLKTYHP - ESLDTAS SKPFLYPNPV - S - GTLYI	SDONOKVEKVOIIYNVL-	GVLVKTSOKG-NESIDL	- G SLASGTYLAKIFTTD - GS ISOTIVKK
Fjoh_1269	GVESRERLAV TAKVNGSL - STDDFVLPN FRY YPNP V - Q - HVLN I	ISNASNIDEVEVISVS-	GKSILVK-Q-INNT-HSEIDL	- SSVSSGLYFLKVKSEG-QSKTIKIVKK
Fjoh_1408	N L P A G E Y R I Y G N K T A N L A - I E D F E K G S T V N L Y P N P V - S - N H F T I	S TAV S K V Q I Y S V S -	GQFVKSFAS-NGNV-DFQFGV	- SELQTGLYIVKASDENGKIQVMKFIKK
Fjoh_1905	VQVESTAKKALSVN - PKENENTADMAVYIDEV - S-DHLKI	ETNH EGTADVEIFNIN-	GQSVLKRNVNFVKGNLSEIEV	- SRLPKGVYIVRVNDGA-GSYSKKVLKQ
Fjoh_2150	<u>V T P P</u> T PG T T F <u>SWT P</u> S T T L - SK PA F N L A E L A V Y P N P V - K - N T L N I	SYQD · · · · · · · KIDNIKIFNVL ·	GQEILNK-N-ISAS-NDTVDM	- SQMLPGTYIAKISANN-IVQTFKIVKI
Fjoh_2389	TPDTSYGYGIPNFGSTLG-TQTFLSTSDFLV YPNP T-K-SNISI	LFDN ETASVSIYSLL -	GOKLIEK-Q-ITNQ-NPVLSV	- EGLTNGLYFYTFDAGS - LH KTGKIIKQ
Fjon_2456	KVNILSGYSTLSVK - ENNKETADISFKVWPVPT - N-GNFSV			- HLREKGVYILKVSNPN-NKKVLHVKKIIVQ
FJON_2000				- SHYAEGIYIIKIKIUV-KTESVKVIKIVR
Fioh 3246			CKAVVKOPKI FINS, NESKEIKT.	
Figh 3324		SKELEWTVESVS-	GSKUKEGR-GNEUSU	- SEOASGIVELKTNASA KALLISKO
Fioh 3731	I GGND FWVVKLKDK - AKVEKVKAS I EAI PNPA - V - TYTN	IIGYDFTEGTASVIDIL-	GRILOOF - SI- IN SR - TV PVDL	- SHYAEGIYIIKIKTDV-KTESVKVIKTVR
Fion_3421	GVQQPYEITTLGN - NEFAEIKLTMTAYPNPV- I - DELSI		GKTVSQNLK-VTTS-ETRVSM	- QGLNQGVYFLVINKNSKNIKTFKIIKK
Fjoh_3777	GPLEPIGPIE - GRSAGNQTSYKIYPNPS - S-NIIN	INLADENYR PV SS SLIRAELYN I S-	GDLKSAV-T-IKNH-TAQLDV	- SALPLGVYVLRINVDG-KTESHQVLVK
Fjoh_3855	ΥΕΊΩΚΙ SIMAEALLGT - ΌΩΝΤΙΚSSLCΥLΚQN PV - Q - ΌΝ LVI	ELAE EYKNEE TLLKIYNTS -	GVLLKE SSYR - PEGLSV	- SDLSQGIYFLSVNNNG-AS KKIKFIKK
Fjoh_4051	DGKVLQTCSG-RITLTNSLQAKLYPNPIQT-GKAI	TVEAD FPQEELNNMQISLYSVS-	GQLIKTV-Q-SSSA-LTEIQLP	- QTIESNILMVVLETPN-VKKSFKVIVK
Fjoh_4174	WIRITKIGNAAA TA AVAAIIS - AEEKTGENILNI YPNP V - S - DVLS	TTDVTGGKINIIDSQ-	GAVIGS QNAA - ENSLNV	- SNLKQGIYFIVLEKDG - QK TIKRFIKK
Fjon_4175	GIFN I NWIFR I TKA AISG LIAAK - ISAISADK I EISLITVIYPN PIS - E - DITLIFI	SAEVSGANVSIINSEG	GATVSTQKAN-DNSINV	
Figh 4177	WIKITKUGAGLAAKTASVU - TEEAPEETVLNVYPSPV- E-NTLF		GRIVLS KKSN-GNSIDV	
Figh 4242			GONTITS PULSE TUSI	
Fioh 4436	SLNNKEVSLDG - NEVSVEDSDLVVVVPNPT - S-GUES	LOIKR PKSAKATVCIVNLN-	GRVLOKR-N-ULFS-EEROSFEEN	I TGATEGINILI RVDCLE - GM TONLI LKN
Fion 4721	YPKDTC SGLG SHTLANL - GTGNFDSA I FSI YPNPS - N - GHFT	IQLKD SNETSNIEIISIL -	GORVFSQ KNSL - NSSINV	-NNIQKGIYIVRITOGS-KTSSKKIIIN
Fjoh_4723	SQDTCNNLGSKMARPIE - EEVVETSDEIVIFPNPS - D - GNFNI	IGLNNFNFPYSLEIFSFT-	GQKVFEK QNAS-DSIISV	- SYLPSGIYIVKIEKDS-KTTIKKIIIN
Fjoh_4948	ΝΓΤΟ ΓΑΝΤ - ΤΡΝΡΟΤΝΥΟ ΕΤΙ ΣΡΝΡΥ ΣΝ - ΟΝΙΤΙ	ITANA VS SQ SVATCR I YDSS -	GVLKLSF - S - LTNS - YTS I PLRNA	ΙΡSLTTGVYΙ FQI ΤΥΑΝGTV ΚΤΚΝLΑVΝ
RemA	ΤΕΤΩR FAL RYTNKTLGTG - DEENVKDGLLISVKD ΚΤΙΚΝ	TSAK ENIKEVNIFDIT-	GKLIYNK KKVG - NTELSI S	NLQSADQVLLVKVNLENNAQITRKVIFK
Celal_2532	Ĭ <u>QVK</u> TAKDCQGLY-EETIVVQNQLTI YPNP VVN-DILHI	NLKTKKVEEVTIAMYASS-	GAQVLYATYPVVNN-VVEVNV	- AKLPKGVFYMRVDLRN - EA LNFKILK
CHU_1335	TINV SVSVGTPTG - VATML SQ SDIEI YPNPT - S - GNSNI	EFKGTFDNIEVTIYSID-	GSKISVSQLGSASSSVGTVEV	- QELPSGVYLVEVNTTO-GKLIKRLIKE
PGN_1466	A DE TINILITIL TV VIGIYNIKIV TV TIK DIVIKIVIE - IGIT SITAD VAIND KIPIYITVIAVISIGI - KITTT	71E SIPIA IAGL THIF DMNI-	IGIR RIVATIA KINIR MIVIFI	EIAIONGIVIYIAVR HAITEIGI-IKTI IYTEKIVIIIVIKI

L

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).

SprB	DGGTIQPGNIFTNIPAC	DHTIRVRHTNG C	TADVDFNIIGYA	PLQLTL	TEEKGVWNVITASAVGGG
Fjoh_1123	NDTDIVSNTYEADLINA	KYTLTVGKIQNDIYC	ENKFEFELVRSVLPTI	[KQ IRYQEL	SDNNFIEII PTTD
Fjoh_1645		NVPLSG	ITITDLLP-GVV	V S GQAL	DLNVGESNDTN
Fjoh_1720	GMSYLWSNGATTQTTDI TKAC	TYTVDVTSPSPENCT	SRKTII VDEHYF	<u>PE</u> INRI	IINGTQVEI QLKKEE
Fjoh_1985		ECSFHL	FAYPVLVDAGED I	E INEGQF	VKLQAV ALENGS
Fjoh_2273	VAGC TSE PA	EVPVTIND	SPVPVLNSDGQNFCGI	L TNPTIGDL SNNTN	IPATVVWYD APDNGN
Fjoh_3478	TPNFGEHVTFTI TVNNV(EGSFIN	TIVSEILPSGYDLVSE	N TTGGTY	DPATQLWTI PALASG
Fjoh_3952		NVPLHN	ITISDLLP-GVV	. I TGG <u>P I</u>	SLGVGESDSHT
Fjoh_3971	EVDQTDAAMGSEVIFTITAENLO	NLTATN	VEVODILPKGYLINSS	ST V SSGTY	NSSTSVWAL PSVNAG
Fjoh_4538		TSNITSEACNDDTTL	LNLSNLLPEGTP	· I TGTWF	DTNDTRALQGN
Fjoh_4750	F SVTD S FGCKAE VAYNVNTPVLC	TAN FISTOSYG	KDMYDLYSI-YDPITH	TN LATGDF	TLI SWDFGD GNFSNE
Fjoh_4934	SYLWSNGETSNSAIISAPC	DYSVTVKDANGCEKT	KNFKVILSEPATITN -	AAVKDF	SGNDNSVLI EYTGT
-					
SprB	GEYVY - SIDGVNFSSETKFKIYF	TGT YTI TVRDKN	GCTDTKDYYIEYVD	VCLDNYFTPN	GDGVNDTWGPGCTN-
Fjoh_1123	GSLEY-SIDGINYONSNYFSNIC	GGTYVVYLRDKE	GCGQDSKEVTV	I DYPK F F TP N	NDGYNDFWHIKNTS-
Fjoh_1645	FSALY-KITOTDINSGKVSNOAS	VOGKSARGVVVEDNS	DYENIDGDK PTVLDLN	IGCK I KVLNAFSPN	GDOKNERFY IOGL E -
Fjoh_1720	PYFEY SVDGINFQDSNIFYDVI	GGL HTAYVKEKN	GCGGIGLDFVV	LV FPAFFTP N	NDSYNDLWEVTGM E -
Fjoh_1985	FSWSP-SAGLNNTKVGNPIA	PQETTTYTVVFTNKE	GCOAEDSVTITVIPLE	EK DETKYGFSPN	GDGINDFWEIDKI T-
Fjoh_2273	LL SASTRUTEQGRYYGFNFPNSA	CFSSEYIEVTVA-L1	DCDNVPND	FFIPDGFSPN	GDGINDSFVIKDI EF
Fjoh_3478	OSLVL-TIVAEVLPSGNYLNVAA	IEISTPLDVDAANNS	ASASVEPIC	LTVYNEFTPN	NDGANDLFRIDCI E -
Fjoh_3952	FTGTY - TLTQAD INAGTV VNQ A	VTGTTQSGIKVEDKS	DAANENGDAPTEIDV	GCKIKIFNAISLN	GDNMNERFY IRGI E -
Fjoh_3971	STOTL TINAKVVDFNDYLNTA	LVKMDOIDTNSSNNO	DSAAVSPNC	LKIYNEFSPN	DDGQNDTFYIDCI T -
Fjoh_4538	ILNAH - GLALGNYQFEYKITNEN	CPRS - ILLTMEVND	DCKVLACEN	· I LVHNAFSPN	GDGKNDVFLIDGIGDLT-
Fjoh_4750	ENPKH - IYTKVGTYTIKQTVTYI	FGCOYSYSATIKVEK	<u>GYS</u>	LIMPNAFTPN	NDGYNDTFAPVFL
Fjoh_4934	GNYEF-SLDGLTFQDNPLFTAVA	TGT YNATAKDKN	GCGLSNSFLLYV	· LDYPRFFTPN	GDGYNDLWVIEDS N -
-					
SprB	IYNHLKFSIFDRYGRVI - AKYT	GQK WDGRYNGE E	L L PSGDYWY	/LKLNDENDG	- REFVGHFTLYR
Fjoh_1123	KFPNSKISIFDRYGKLIKELFA	DHG WDGFYNGSQ	MPADDYWFF	ANF NEN	- INFSGHFSLKR
Fjoh_1645	CYPENTVEIYNRWGVLVFDVDHY	NN V DRVFKGY S FGRT	TMKQSEGLPVGTYFYI	ILKYKDSDSN	PHETSGYLYINK
Fjoh_1720	NYPQAQVTIFDRYGKLIAQLNAS	KMS WDGTFEKTP	MPASDYWY	LKIDDS	KPILRGHFSLKR
Fjoh_1985	DYPENEVLIYSRWGDLVYQTKG	DNS TNVFSGIANKSR	NLGASQ - LPEGTYFFE	EIRVNOPHHF	- KKLKGYLVLKR
Fjoh_2273	LYPNYTLEIFNRYGNGMYKGDKI	IKPA WDGMNYEKS	GIAGGV - APNGVYFYN	/LHFN KDN	KP PKQGRLY LNR
Fjoh_3478	SYPNNELKVFNRYGALVYSKQH	'END WDG TANV SG	VVNRGDMLPTGTYFY	/ I TT GDG	- TVKKGWLSIMR
Fjoh_3952	CYPDNTVQIFNRWGVLVFERDHY	NNND I VFKGFSEGRT	TVKESNGLPEGTYYYI	VRYKONNSN	PKQEAGYLYLIK
Fjoh_3971	QYPDNQLEIFNRWGNLVYYKKGY	DNT WDGKADGSA	KTLPEGTYFY	/LDL GNG	SAKKSGWLYL-K
Fjoh_4538	CYPENTVEIYNRWGILVFETHNY	NNTTNAFD GT S RGRT	TIRQSEGLPTGTYFYI	VTYKSVDGNNVIO	NNKKEGYLYLSK
Fjoh_4750	GLSDITLDVFDTWGGVIYTEKG	NIRG WINGKVKDID	AENGNYYY	IIIKTFYNH	TIVEKGAFTLIK
Fjoh_4934	VLPNYTIHI FDRYGKFLKEMNOT	SPG WNGLFNGQQ		ПLТF ADG	- RNVKGHFSLKR
-					

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 $\Delta gldNO$ 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 $\Delta gldNO$ 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 $\Delta gldNO$ 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.



B



С Exponential phase Stationary phase ∆sprA+pSK97 ∆sprA+pSK97 WT+pSK97 WT+pSK97 WT+pSK97 WT+pSK97 +pSN48 +pSN48 Whole cells Spent media Spent media Spent media Spent media Whole cells Whole cells Spent media Whole cells Spent media Whole cells Whole cells kDa 56 43 34 26

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 $\Delta sprA$ 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- μ m 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 μ m.

A



В



Fig. S8. Detection of cell-surface localized SprB. Protein G-coated 0.5- μ m 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 μ m.

Strain	Source or		
		reference	
<i>E. coli</i> strains			
DH5amer	Strain used for general cloning	Life	
		Technologies	
		(Grand Island,	
110101		NY, USA)	
HBI01	Strain used with pRK2013 for triparental conjugation	(1, 2)	
F. johnsoniae			
strains	wild true E is huge wing ATCC 170(1 ^T	(2)	
FJI CU1927	wild type F. Johnsoniae ATCC 1/001	(3)	
CJ1827	construction of deletion mutants	(4)	
CJ2122	$\Delta g l d K$	(5)	
CJ2157	$\Delta g l d L$	(5)	
CJ2262	$\Delta g l d M$	(5)	
CJ1631A	$\Delta(gldN-gldO)$	(6)	
CJ2302	$\Delta sprA$	(5)	
FJ149	sprE	(7)	
CJ2518	$\Lambda sprF$	(8)	
KDF002	sprT	(9)	
CJ2116	$\Delta porU$	(10)	
CJ2130	$\Delta porV$	(10)	
CJ1922	$\Delta sprB$	(4)	
CJ1984	$\Delta rem A$	(11)	
FJ117	<i>sprB HimarEm2</i> mutant	(12)	
FJ156	sprB HimarEm2 mutant	(12)	
		0	
Plasmid	Description	Source or	
		reference	
nCB3	735-bn sfGEP without stop codon amplified and cloned	This study	
ревз	into nSSK52 Encodes SPCHA-sfGEP-CTDCHA(105AA) ^c An ^r	This study	
	(Tc^{r})		
pCB4	440-bp region encoding 62 amino acids of CTD_{ChiA}	This study	
r -	inserted into pCB3. Encodes SP _{ChiA} -sfGFP-CTD _{ChiA} (62AA);	j	
	$Ap^{r}(Tc^{r})$		
pCP11	<i>E. coli-F. johnsoniae</i> shuttle plasmid; Ap ^r (Em ^r)	(13)	
pCP23	<i>E. coli-F. johnsoniae</i> shuttle plasmid; Ap ^r (Tc ^r)	(14)	
pMM105.A	E. coli-Capnocytophaga canimorsus shuttle plasmid;	(15)	
	$Ap^{r}(Em^{r})$		
pRK2013	Helper plasmid for triparental conjugation; IncP Tra ⁺ Km ^r	(2)	
pRR48	1294-bp fragment spanning <i>sprF</i> inserted into pCP23;	(16)	

	$Ap^{r}(Tc^{r})$	
pSK30	339-bp region encoding 97 amino acids of CTD _{RemA}	This study
	inserted into pYT179. Encodes SP _{RemA} -sfGFP-	
	$CTD_{RemA(97AA)}; Ap^{r} (Tc^{r})$	
pSK37	SP _{RemA} -sfGFP with stop codon cloned into pYT40.	This study
	Encodes SP-sfGFP; Ap ^r (Tc ^r)	
pSK56	657-bp region encoding 218 amino acids of CTD _{SprB}	This study
	inserted into pYT179. Encodes SP-sfGFP- CTD _{SprB(218AA)} ;	
	$Ap^{r}(Tc^{r})$	
pSK58	687-bp region encoding 228 amino acids of CTD _{Fjoh_3952}	This study
	inserted into pYT179. Encodes SP-sfGFP-	
	$CTD_{Fjoh_3952(228AA)}; Ap^r (Tc^r)$	
pSK62	3549-bp region encoding 1182 amino acids of CTD _{SprB}	This study
	inserted into pYT179. Encodes SP-sfGFP-	
	$CTD_{SprB(1182AA)}; Ap^{r} (Tc^{r})$	
pSK65	417-bp region encoding 108 amino acids of CTD _{Celal_2532}	This study
	inserted into pYT179. Encodes SP-sfGFP- CTD _{Celal_2532} ;	
	$Ap^{r}(Tc^{r})$	
pSK71	312-bp region encoding 87 amino acids of CTD _{RemA}	This study
	inserted into pYT179. Encodes SP-sfGFP- CTD _{RemA(87AA)} ;	
	$Ap^{r}(Tc^{r})$	
pSK75	339-bp region encoding 103 amino acids of CTD _{PGN_1466}	This study
	inserted into pYT179. Encodes SP-sfGFP- CTD _{PGN_1466} ;	
	$Ap^{i}(Tc^{i})$	
pSK76	294-bp region encoding 97 amino acids of CTD _{CHU_1335}	This study
	inserted into pYT179 Encodes SP-sfGFP- CTD _{CHU_1335} ;	
~~~~	$Ap^{r}(Tc^{r})$	
pSK79	258-bp region encoding 85 amino acids near the C-	This study
	terminus of RemA but lacking the C-terminal 12 amino	
	acids inserted into pY11/9. Encodes SP-stGFP-	
CIZ 01	$C I D_{\text{RemA}(\text{lacking final 12 AA})}; Ap^{-} (Ic^{-})$	TT1 · / 1
ръкът	234-bp region encoding 62 amino acids of $C I D_{RemA}$	This study
	Inserted Into p Y 11/9. Encodes SP-SIGPP- C I $D_{\text{RemA}(62AA)}$ ,	
-CV02	Ap $(10)$	This study
psk82	inserted into pVT170. Encodes SD sfCED. CTD	This study
	Inserted into p 1 11/9. Encodes SF-SIOFF- C 1 $D_{\text{AmyB}(99AA)}$ , $\Delta p^{\text{r}} (\text{T} p^{\text{r}})$	
nSK81	Ap (10) 306 hn fragment spanning the Figh 1634 promoter start	This study
p3K04	codon, and the N terminal signal pentide encoding region	This study
	inserted into pSK30. Encodes SPress was sfGEP_CTD	
	$\Delta n^{r} (Tc^{r})$	
nSK85	312-bn region encoding 73 amino acids of CTD	This study
Poixos	inserted into nYT179 Encodes SP-sfGFP- CTD	This study
	$An^{r}(Tc^{r})$	
pSK86	270-bp region encoding 59 amino acids of $CTD_{AmuB}$	This study
r	inserted into pYT179. Encodes SP-sfGFP- CTD _{AmvB(59AA)} ;	

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

^{*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' GCTAG <u>GGTACC</u> ACGTTCCTGATAGGCACAAAAATGC 3'; forward
	primer used in construction of pYT40; KpnI site underlined
1270	5' GCTAG <u>GGATCC</u> GCCATTAGTTGGCATTCCAGGAAAA 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' GCTAG <u>TCTAGA</u> GATCGTTTTGCACTTCGTTACACT 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' GCTAG <u>TCTAGA</u> GCAACGATAGCTTATTTTAAAAACAAT 3'; forward
	primer used in construction of pSK89; XbaI site underlined
1600	5' GCTAG <u>TCTAGA</u> GCTTATGCAGCTTATTTCGCATCACAA 3'; forward
	primer used in construction of pSSK52; XbaI site underlined
1771	5' GCTAG <u>GGATCC</u> CTAACCCGACTATCATAGAACCGAC 3'; forward
	primer used in construction of pYT314; BamHI site underlined
1772	5' GCTAG <u>GTCGAC</u> TGTTGTTACAGCCATGAGTACTAAGG 3'; reverse
	primer used in construction of pYT314; SalI site underlined
1773	5' GCTAGGTCGACTCGATTAGTAACTGTCCTTGTACGC 3'; forward primer
	used in construction of pYT316; Sall site underlined
1774	5' GCTAG <u>GCATGC</u> TAAAAGTTCAGTTGGCAGTTCTTCG 3'; reverse primer
	used in construction of pYT316; SphI site underlined
1880	5' GCTAG <u>GCATGC</u> TGGCGAGGAATTACCTTCTGGTGA 3'; forward primer
	used in construction of pSK62; XbaI site underlined
1843	5' GCTAG <u>TCTAGA</u> GTGGTGATTACAATTGATCCAAGC 3'; forward primer
	used in construction of pSK56; XbaI site underlined
1868	5' GCTAG <u>TCTAGA</u> GTCGAAGTGCCATCGATTACAGTA 3'; forward primer
	used in construction of pSK58; XbaI site underlined
1885	5' GCTAG <u>TCTAGA</u> GCTTTAGAGGCTTTTGAAAATGTG 3'; forward primer
	used in construction of pSK65; XbaI site underlined
1886	5' GCTAG <u>GCATGC</u> TTGTGGGCGTTTCTGAACTATCTC 3'; reverse primer
	used in construction of pSK65; SphI site underlined

1870	5' GCTAG <u>GCATGC</u> GCTAAGCCATTTTATTGATTTGGA 3'; reverse primer used in construction of pSK 58: SphI site underlined
1899	5' GCTAG <u>TCTAGA</u> ACATTAGGAACTGGTGATTTTGAG 3'; forward primer
1923	5' GCTAG <u>TCTAGA</u> GAGAGTATCGCTGATGAAACGAAC 3'; forward primer
1924	5' GCTAG <u>GCATGC</u> GCCCTTATTAGAGAATTGCAGTGT 3'; reverse primer
1925	used in construction of pSK/5; Sph1 site underlined 5' GCTAG <u>TCTAGA</u> GTATCGGTAAGTGTGGGAACTCCT 3'; forward primer
1926	used in construction of pSK76; Xbal site underlined 5' GCTAG <u>GCATGC</u> CTGTATAGGCTATTCTTTTATAAGGCG 3'; reverse
1930	primer used in construction of pSK76; SphI site underlined 5' GCTAGTCTAGAACTTCTGCAAAAGAAATATTAAAGAA 3'; forward
1000	primer used in construction of pSK81; XbaI site underlined
1932	5' GCTAG <u>GCATGCCTATTCAAGATTAACTTTTACAAGCAGCAC 3';</u> reverse primer used in construction of pSK79: SphI site underlined
1933	5' GCTAG <u>TCTAGA</u> GAACCAACAACTGTTGGAACAGGA 3'; forward primer
1934	5' GCTAG <u>GCATGC</u> CGAATCGAACAATAGCGAACAAGC 3'; reverse primer
1940	used in construction of pSK82; SphI site underlined 5' GCTAGTCTAGAGAAGACATTGCTCAGGTTGATGTA 3'; forward primer
1046	used in construction of pCB4; XbaI site underlined
1946	used in construction of pSK84; KpnI site underlined
1947	5' GCTAG <u>GGATCC</u> ATCTTGAGCAAATGAAGTTAGGGA 3'; reverse primer used in construction of pSK 84: BamHI site underlined
1948	5' GCTAG <u>TCTAGA</u> TATCCAAACCCATCTGTAAACAATGAA 3'; forward
1949	5' GCTAG <u>TCTAGA</u> CCAGAATTGGAAAGCGGAGAC 3'; forward primer used
1962	in construction of pSK86; XbaI site underlined 5' GCTAG <u>GCATGC</u> CTATTTAAAGATCACTGCTCTGGTTATCTG 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					CTD	s
	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	) (	ı 0	0	0
Elizabethkingia sp. BM10	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 1599	7 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 Sphingobacterila							
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	C	. 0	0	0	0	0	0
Bacteroides cellulosilvticus WH2	1	. 1	1	1	1	19	1
Bacteroides dorei CL03T12C01	0	. 0	0	0	0	0	0
Bacteroides fraailis NCTC 9343	ū	1	1	0	0	0	0
Bacteroides helcogenes DSM 20613	N		-	<u>و</u> ۱	n 0	ů N	0 0
Bacteroides ovatus ATCC 8483	0	i î	0 0	0	n N	ů n	0 0
Bacteroides thetaiotaomicron VPI-5482	0 N	i î	ů N	0	0 0	ů N	0 0
Bacteroides vulgatus ATCC 8487	0	i î	0	0	0 0	0 0	ů Ú
Bacteroides vulgatus Arec 6462	0	, 0 , 0	0	0	0	0	0
Barparialla viscaricala DSM 19177	1	1	1	1	1	41	1
Draconibactorium orientale EUE	-	. 1	1	·			
Oderiberter enlandbridge DEM 20712					1	23	1
Dabribacter spianchinicus DSW 20712	1	· U	0	1	1	2	
Parabarbarbarbidas distancesis ATCC 8502	1	. 1	1		1	10	0
Parabacterolaes aistasonis ATCC 8503	L	. 1	1	. 1	1	/	1
Porphyromonas asaccharolytica DSM 20707	1	. 1	1	. 1	1	29	1
Porphyromonas gingivalis AICC 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					СТД	s
	GldK	GldL	GldM	GldN	SprA	Type A CTD	Type B CTD
	TIGR03525	TIGR03513	TIGR03517	TIGR03523	TIGR04189	TIGR04183	TIGR04131

^aOnly 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
<i>Ignavibacterium album</i> 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

^a3777 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.

## References

- 1. **Bolivar F, Backman K.** 1979. Plasmids of *Escherichia coli* as cloning vectors. Methods Enzymol. **68**:245-267.
- 2. **Figurski DH, Helinski DR.** 1979. Replication of an origin-containing derivative of plasmid RK2 dependent on a plasmid function provided in trans. Proc. Natl. Acad. Sci. USA **76:**1648-1652.
- Braun TF, Khubbar MK, Saffarini DA, McBride MJ. 2005. Flavobacterium johnsoniae gliding motility genes identified by mariner mutagenesis. J. Bacteriol. 187:6943-6952.
- 4. **Rhodes RG, Pucker HG, McBride MJ.** 2011. Development and use of a gene deletion strategy for *Flavobacterium johnsoniae* to identify the redundant motility genes *remF*, *remG*, *remH*, and *remI*. J. Bacteriol. **193:**2418-2428.
- 5. Shrivastava A, Johnston JJ, van Baaren JM, McBride MJ. 2013. *Flavobacterium johnsoniae* GldK, GldL, GldM, and SprA are required for secretion of the cell-surface gliding motility adhesins SprB and RemA. J Bacteriol **195**:3201-3212.
- 6. Rhodes RG, Samarasam MN, Shrivastava A, van Baaren JM, Pochiraju S, Bollampalli S, McBride MJ. 2010. *Flavobacterium johnsoniae gldN* and *gldO* are partially redundant genes required for gliding motility and surface localization of SprB. J. Bacteriol. **192:**1201-1211.
- 7. **Rhodes RG, Samarasam MN, Van Groll EJ, McBride MJ.** 2011. Mutations in *Flavobacterium johnsoniae sprE* result in defects in gliding motility and protein secretion. J Bacteriol **193:**5322-5327.
- 8. **Zhu Y, Thomas F, Larocque R, Li N, Duffieux D, Cladiere L, Souchaud F, Michel G, McBride MJ.** submitted. Novel genetic tools unravel the crucial role of a laterally acquired alginate lyase for brown algal biomass degradation by *Zobellia galactanivorans*
- 9. Sato K, Naito M, Yukitake H, Hirakawa H, Shoji M, McBride MJ, Rhodes RG, Nakayama K. 2010. A protein secretion system linked to bacteroidete gliding motility and pathogenesis. Proc. Natl. Acad. Sci. USA 107:276-281.
- Kharade SS, McBride MJ. 2015. *Flavobacterium johnsoniae* PorV is required for secretion of a subset of proteins targeted to the type IX secretion system. J. Bacteriol. 197:147-158.
- Shrivastava A, Rhodes RG, Pochiraju S, Nakane D, McBride MJ. 2012. *Flavobacterium johnsoniae* RemA is a mobile cell-surface lectin involved in gliding. J. Bacteriol. 194:3678-3688.
- 12. **Nelson SS, Bollampalli S, McBride MJ.** 2008. SprB is a cell surface component of the *Flavobacterium johnsoniae* gliding motility machinery. J. Bacteriol. **190:**2851-2857.
- 13. **McBride MJ, Kempf MJ.** 1996. Development of techniques for the genetic manipulation of the gliding bacterium *Cytophaga johnsonae*. J. Bacteriol **178**:583-590.
- 14. **Agarwal S, Hunnicutt DW, McBride MJ.** 1997. Cloning and characterization of the *Flavobacterium johnsoniae* (*Cytophaga johnsonae*) gliding motility gene, *gldA*. Proc. Natl. Acad. Sci. USA **94**:12139-12144.
- 15. **Mally M, Cornelis GR.** 2008. Genetic tools for studying *Capnocytophaga canimorsus*. Appl Environ Microbiol **74:**6369-6377.
- 16. **Rhodes RG, Nelson SS, Pochiraju S, McBride MJ.** 2011. *Flavobacterium johnsoniae sprB* is part of an operon spanning the additional gliding motility genes *sprC*, *sprD*, and *sprF*. J. Bacteriol. **193:**599-610.

- Nelson SS, Glocka PP, Agarwal S, Grimm DP, McBride MJ. 2007. Flavobacterium johnsoniae SprA is a cell-surface protein involved in gliding motility. J. Bacteriol. 189:7145-7150.
- Kharade SS, McBride MJ. 2014. The *Flavobacterium johnsoniae* chitinase ChiA is required for chitin utilization and is secreted by the type IX secretion system. J. Bacteriol. 196:961-970.
- 19. Uehara T, Dinh T, Bernhardt TG. 2009. LytM-domain factors are required for daughter cell separation and rapid ampicillin-induced lysis in *Escherichia coli*. J Bacteriol **191:**5094-5107.
- 20. Veith PD, Nor Muhammad NA, Dashper SG, Likic VA, Gorasia DG, Chen D, Byrne SJ, Catmull DV, Reynolds EC. 2013. Protein substrates of a novel secretion system are numerous in the *Bacteroidetes* phylum and have in common a cleavable C-terminal secretion signal, extensive post-translational modification and cell surface attachment. J. Proteome Res. 12:4449-4461.