

**Supplemental Figure and Tables for:**

SS Kharade and MJ McBride. "*Flavobacterium johnsoniae* PorV is required for secretion of a subset of proteins targeted to the type IX secretion system"

Table S1. Primers used in this study

Primers	Sequence and Description
737	5'-AGGCACCCCAGGCTTTACACT-3'; Reverse primer binding downstream of multiple cloning site of pLYL03.
948	5' - GCTAGGGATCCTCTTGCAGGCTCAACTACCGGAAT - 3'; Forward primer to construct pSSK01; BamHI site underlined
949	5' - GCTAGGTCGACCTTTTCGCTTGCCAAACCGTCTTCA - 3'; Reverse primer to construct pSSK01; Sall site underlined
972	5' - GCTAGGCATGC TACGTGGGACGGAAGGGATGATTT - 3'; Forward primer used for constructing pSSK03; SphI site underlined
973	5' - GCTAGGGTACCCCTGAGAAAGCTCATTGATGGTGTCTG - 3'; Reverse primer to construct pSSK03; KpnI site underlined
988	5' - GCTAGTCTAGAGCCGTGCCCAACATCAATACCATT - 3'; Forward primer used for constructing pSSK04; XbaI site underlined
989	5' - GCTAGGGATCCAGTTGCAACACCCTGGTCTCCTAA - 3'; Reverse primer to construct pSSK04; BamHI site underlined
1102	5' - GCTAGGGATCCGCAAACACTTCGAACTTCGTA - 3'; Forward primer to amplify upstream region of <i>Fjoh_0288</i> to construct pSSK16; BamHI site underlined
1103	5' - GCTAGTCTAGAGGCATCGACACCAATATTCAT - 3'; Reverse primer to amplify upstream region of <i>Fjoh_0288</i> to construct pSSK16; XbaI site underlined
1104	5' - GCTAGTCTAGAGCTTTGTACTCAAATATTTTTTCGATA - 3'; Forward primer to amplify downstream region of <i>Fjoh_0288</i> to construct pSSK14; XbaI site underlined
1105	5' - GCTAGGTCGACCAGTTTTATCGCCTGAGCTTC - 3'; Reverse primer to amplify downstream region of <i>Fjoh_0288</i> to construct pSSK14; Sall site underlined
1201	5' - GCTAGGTCGACTACCACGAAAGCCCTATGAAAGGA - 3'; Forward primer to amplify downstream region of <i>porV</i> to construct pSSK22; Sall site underlined
1202	5' - GCTAGGCATGCCTCCTTCAGCTACTGTATCACCAAC-3'; Reverse primer to amplify downstream region of <i>porV</i> to construct pSSK22; SphI site underlined
1203	5' - GCTAGGGATCCTGCCATTGATGCGTCTGACTAC - 3'; Forward primer to amplify upstream region of <i>porV</i> to construct pSSK20; BamHI site underlined
1204	5' - GCTAGGTCGACAGGGCGCTCAATATCTTGGGCTTT - 3'; Reverse primer to amplify upstream region of <i>porV</i> to construct pSSK20; Sall site underlined
1205	5' - GCTAGGTCGACGATTTTGGCGACCGGATTGGAAAAGGA - 3'; Forward primer to amplify downstream region of <i>porU</i> to construct pSSK23; Sall site underlined
1206	5' - GCTAGGCATGCCCATACCAATTGGCTGAACGTGGT - 3'; Reverse primer to amplify downstream region of <i>porU</i> to construct pSSK23; SphI site underlined
1207	5' - GCTAGGGATCCCGACAGTTCCTGCAGTGTTCCTAAGC-3'; Forward primer to amplify upstream region of <i>porU</i> to construct pSSK21; BamHI site underlined
1208	5' - GCTAGGTCGACAAAGTATGCGATCAGGGCTTGTTTCAT - 3'; Reverse primer to amplify upstream region of <i>porU</i> to construct pSSK21; Sall site underlined

Table S2. Bacteriophage sensitivity of *F. johnsoniae* wild-type and mutant strains<sup>a</sup>.

Phage Host Strain	φCj1 pfu/ml	φCj13 pfu/ml	φCj23 pfu/ml	φCj28 pfu/ml	φCj29 pfu/ml	φCj42 pfu/ml	φCj48 pfu/ml	φCj54 pfu/ml
WT (CJ1827)	1.5 x 10 <sup>8</sup>	1.6 x 10 <sup>8</sup>	1.5 x 10 <sup>8</sup>	1.7 x 10 <sup>9</sup>	1.5 x 10 <sup>9</sup>	1.7 x 10 <sup>9</sup>	1.7 x 10 <sup>9</sup>	1.7 x 10 <sup>9</sup>
<i>ΔporU</i>	1.5 x 10 <sup>8</sup>	1.5 x 10 <sup>8</sup>	1.5 x 10 <sup>8</sup>	1.7 x 10 <sup>9</sup>	1.4 x 10 <sup>9</sup>	1.6 x 10 <sup>9</sup>	1.6 x 10 <sup>9</sup>	1.7 x 10 <sup>9</sup>
<i>ΔporV</i>	1.4 x 10 <sup>8</sup>	1.4 x 10 <sup>8</sup>	1.5 x 10 <sup>8</sup>	1.7 x 10 <sup>9</sup>	1.1 x 10 <sup>9</sup>	1.3 x 10 <sup>9</sup>	< 10	< 10
<i>ΔporV</i> + pSSK03	1.5 x 10 <sup>8</sup>	1.5 x 10 <sup>8</sup>	1.5 x 10 <sup>8</sup>	1.7 x 10 <sup>9</sup>	1.4 x 10 <sup>9</sup>	1.6 x 10 <sup>9</sup>	1.6 x 10 <sup>9</sup>	1.7 x 10 <sup>9</sup>
<i>ΔremA</i>	1.5 x 10 <sup>8</sup>	1.5 x 10 <sup>8</sup>	1.5 x 10 <sup>8</sup>	1.7 x 10 <sup>9</sup>	1.5 x 10 <sup>9</sup>	1.5 x 10 <sup>9</sup>	1.5 x 10 <sup>9</sup>	1.6 x 10 <sup>9</sup>
<i>ΔsprB</i>	< 10	1.2 x 10 <sup>3</sup>	< 10	1.7 x 10 <sup>9</sup>	1.0 x 10 <sup>9</sup>	1.7 x 10 <sup>9</sup>	1.6 x 10 <sup>9</sup>	1.7 x 10 <sup>9</sup>
<i>ΔremA ΔsprB</i>	< 10	1.1 x 10 <sup>3</sup>	< 10	1.7 x 10 <sup>9</sup>	1.0 x 10 <sup>9</sup>	1.4 x 10 <sup>9</sup>	1.5 x 10 <sup>9</sup>	1.6 x 10 <sup>9</sup>
<i>ΔporV ΔsprB</i>	< 10	< 10	< 10	1.7 x 10 <sup>9</sup>	< 10	< 10	< 10	< 10
<i>ΔgldNO</i>	< 10	< 10	< 10	< 10	< 10	< 10	< 10	< 10

<sup>a</sup> Each phage stock was serially diluted, mixed with cells of *F. johnsoniae*, plated on CYE agar in CYE overlay agar, incubated 24 h at 25°C, and the number of plaque forming units (pfu) per ml of original phage stock was determined on each bacterial strain. '<10' indicates that no plaques were observed even when 0.1 ml of undiluted phage stock was tested. 'WT' indicates the streptomycin resistant *rpsI2* strain CJ1827, which is wild-type for secretion and motility and was used to construct deletion mutants. *ΔporU*, CJ2116. *ΔporV*, CJ2130. *ΔremA*, CJ1984. *ΔsprB*, CJ1922. *ΔremA ΔsprB*, CJ1985. *ΔporV ΔsprB*, CJ2445. *ΔgldNO*, CJ2090. pSSK03 carries *porV* and was used to complement the *ΔporV* mutant.

Table S3. Proteins identified in cell-free culture fluid by SDS-PAGE followed by LC-MS/MS analysis of the 60-240 kDa range<sup>a</sup>.

<b>Proteins secreted by the T9SS: These 33 proteins were absent or greatly decreased in culture fluid of <i>ΔgldNO</i> mutant cells and were restored by complementation with pTB79.</b>									
Locus tag/Protein name	CTD <sup>b</sup>	Predicted protein function <sup>c</sup>	Wild type	<i>ΔgldNO</i>	<i>ΔgldNO</i> + pTB79	<i>ΔporV</i>	<i>ΔporV</i> + pSSK03	<i>ΔporU</i>	<i>ΔporU</i> + pSSK04
Fjoh_0074	TIGR04183	nuclease/phosphatase	42	3	108	7	84	95	129
Fjoh_0601			115	0	84	71	78	118	148
Fjoh_0602			68	0	38	57	59	65	86
Fjoh_0604			47	0	39	42	44	40	45
Fjoh_0606			163	0	172	77	169	176	198
Fjoh_0808/ RemA	TIGR04183	motility adhesin	38	0	47	0	56	37	67
Fjoh_0886	TIGR04183	peptidase	12	0	19	0	21	14	18
Fjoh_1022	TIGR04183	licheninase	6	0	6	0	6	1	8
Fjoh_1123	TIGR04131		34	0	10	27	3	37	32
Fjoh_1188	TIGR04183		49	0	104	0	42	52	75
Fjoh_1189	TIGR04183	lectin	74	0	112	1	69	112	79
Fjoh_1208	TIGR04183	α amylase	45	0	66	6	91	58	126
Fjoh_1231	TIGR04183	pectate lyase	9	0	13	0	6	35	31
Fjoh_1269	TIGR04183		27	4	43	3	40	34	56
Fjoh_1408 <sup>d</sup>	TIGR04183	α amylase	2	0	4	0	2	0	6
Fjoh_1645 <sup>d</sup>	TIGR04131		2	0	6	0	1	2	3
Fjoh_2150	TIGR04183		6	0	6	0	7	3	3
Fjoh_2273	TIGR04131		4	0	5	1	5	5	1
Fjoh_2389 <sup>d</sup>	TIGR04183	peptidase	2	0	7	0	12	0	6
Fjoh_2667			28	0	7	0	3	5	33
Fjoh_2687			26	1	26	7	35	26	43
Fjoh_3108			7	0	10	0	8	0	10
Fjoh_3246	TIGR04183		12	0	77	0	6	23	41
Fjoh_3324	TIGR04183	carbohydrate-binding	16	1	40	5	20	49	59
Fjoh_3729			46	0	32	0	52	23	79
Fjoh_3777	TIGR04183	deacylase	10	0	25	0	9	10	34
Fjoh_3952	TIGR04131		22	0	11	12	16	11	17

Fjoh_4174	TIGR04183	carbohydrate-binding,	40	5	40	6	62	55	36
Fjoh_4176	TIGR04183	carbohydrate-binding,	48	3	65	7	63	108	76
Fjoh_4177	TIGR04183	glycoside hydrolase, GH16	22	0	35	0	34	67	60
Fjoh_4750	TIGR04131		13	0	3	3	3	3	10
Fjoh_4819		glycoside hydrolase, GH3	34	0	5	0	1	12	9
Fjoh_4934	TIGR04131		11	1	7	19	4	17	17

**Proteins that may be secreted by the T9SS but for which the LC/MS data were inconclusive either because the proteins were abundant in the cell-free culture fluid of the  $\Delta$ *gldNO* mutant, or because of lack of complementation by pTB79.**

Locus tag/Protein name	CTD <sup>b</sup>	Predicted protein function <sup>c</sup>	Wild type	$\Delta$ <i>gldNO</i>	$\Delta$ <i>gldNO</i> + pTB79	$\Delta$ <i>porV</i>	$\Delta$ <i>porV</i> + pSSK03	$\Delta$ <i>porU</i>	$\Delta$ <i>porU</i> + pSSK04
Fjoh_0119		peptidase	24	1	2	6	0	3	5
Fjoh_0200			27	3	1	2	2	2	1
Fjoh_0416			449	85	50	104	58	102	114
Fjoh_0488			31	14	1	16	5	4	8
Fjoh_0636		peptidase	89	21	14	29	19	21	29
Fjoh_0660			46	5	0	14	2	2	1
Fjoh_0674		beta-N-acetylhexosaminidase	5	0	0	0	0	0	0
Fjoh_0707	TIGR04183	periplasmic ligand-binding sensor protein	12	2	1	2	4	1	2
Fjoh_0736		SusC-like protein	3	0	5	5	2	1	2
Fjoh_0761			6	0	0	0	0	0	0
Fjoh_0778		glycoside hydrolase	6	0	0	0	0	0	0
Fjoh_0798	TIGR04183	proprotein convertase	19	8	17	12	8	4	19
Fjoh_0846			15	1	0	4	1	0	0
Fjoh_0959		endothelin-converting protein 1	133	52	47	52	44	63	55
Fjoh_0979/ SprB <sup>c</sup>	TIGR04131	motility adhesin	98	25	58	62	58	60	65
Fjoh_1190		peptidase	107	36	14	41	35	26	27
Fjoh_1191		peptidase	108	15	14	26	21	23	33
Fjoh_1209		Glycoside hydrolase	29	0	1	4	1	1	3
Fjoh_1313		prolyl oligopeptidase	162	56	60	65	44	78	74
Fjoh_1348		peptidase	18	0	0	0	0	0	0
Fjoh_1399		alpha amylase	14	0	0	0	0	2	1
Fjoh_1400			41	3	4	6	2	5	5

Fjoh_1430		carboxyl-terminal protease	85	28	0	32	12	12	24
Fjoh_1434			27	0	0	0	0	0	0
Fjoh_1543		peptidase	50	9	0	22	4	0	2
Fjoh_1564		glycoside hydrolase	30	0	0	2	0	3	13
Fjoh_1567		glycoside hydrolase	76	8	3	17	3	14	32
Fjoh_1773		aminopeptidase	104	21	9	25	14	22	37
Fjoh_1993		alpha-glucuronidase	14	0	0	0	0	0	0
Fjoh_2039		beta-N-acetylhexosaminidase	26	0	1	1	0	0	1
Fjoh_2041		alpha-1,2-mannosidase	12	0	0	0	0	0	1
Fjoh_2042		glycoside hydrolase	35	0	1	1	1	4	1
Fjoh_2253		peptidase	73	8	5	18	3	18	14
Fjoh_2585			11	2	1	3	3	1	2
Fjoh_2592		OstA family protein	18	1	0	3	0	0	3
Fjoh_2593		alpha amylase	6	0	0	0	0	0	0
Fjoh_2612			20	1	0	2	0	1	2
Fjoh_2668			44	0	0	0	51	0	76
Fjoh_2713		glycoside hydrolase	9	0	0	0	0	0	1
Fjoh_2715		alpha-1,2-mannosidase	20	0	0	0	0	0	1
Fjoh_2867		peptidase	49	24	19	22	13	36	29
Fjoh_2928		peptidase	29	1	0	0	1	2	3
Fjoh_2961		LmbE family protein	49	0	1	5	6	2	2
Fjoh_3112			12	0	0	1	0	1	2
Fjoh_3113		alpha-N-arabinofuranosidase	10	0	0	0	0	0	0
Fjoh_3389		glycoside hydrolase	15	4	0	3	2	0	0
Fjoh_3392		glycoside hydrolase	76	14	11	38	9	24	42
Fjoh_3437		aminopeptidase N-like protein	40	2	0	3	1	1	1
Fjoh_3861		glycoside hydrolase	33	0	0	1	0	0	1
Fjoh_3883		glycoside hydrolase	21	0	0	0	0	0	0
Fjoh_4076			5	0	0	0	0	0	0
Fjoh_4080		alpha-L-rhamnosidase	24	0	0	0	0	0	0
Fjoh_4085		alpha-L-rhamnosidase	5	0	0	0	0	0	0
Fjoh_4086			7	0	0	0	0	0	1
Fjoh_4097			18	0	0	0	0	0	0
Fjoh_4098			9	0	0	0	0	0	0
Fjoh_4198		glycoside hydrolase	14	0	0	1	0	0	0
Fjoh_4200		aldehyde oxidase and	15	0	1	2	0	0	7

		xanthine dehydrogenase							
Fjoh_4429			34	0	0	4	0	0	5
Fjoh_4430		alpha-glucosidase	11	0	0	0	0	0	1
Fjoh_4555/ ChiA <sup>T</sup>		chitinase	34	18	308	21	103	491	440
Fjoh_4558		SusD-like protein	12	6	9	3	8	39	42
Fjoh_4602		peptidase	7	0	0	0	1	0	2
Fjoh_4808		beta-N- acetylhexosaminidase	103	9	3	13	3	3	9
Fjoh_4809			6	0	0	0	0	0	0
Fjoh_4868		aminopeptidase N-like protein	52	5	4	12	7	10	17
Fjoh_4963		glycoside hydrolase	15	1	0	1	1	0	2
Fjoh_5032		prolyl oligopeptidase	60	21	9	29	16	26	23

**Proteins that were apparently not secreted by the T9SS: These proteins were not greatly decreased in culture fluid of  $\Delta gldNO$  mutant cells.**

Locus tag/Protein name	CTD <sup>b</sup>	Predicted protein function <sup>c</sup>	Wild type	$\Delta gldNO$	$\Delta gldNO$ + pTB79	$\Delta porV$	$\Delta porV$ + pSSK03	$\Delta porU$	$\Delta porU$ + pSSK04
Fjoh_0024		threonyl-tRNA synthetase	13	26	8	30	4	6	13
Fjoh_0099		carbamoyl phosphate synthase large subunit	1	8	0	10	0	0	0
Fjoh_0112		phosphoglucomutase	7	17	2	12	1	9	9
Fjoh_0184		SusD-like protein	0	0	0	0	0	2	6
Fjoh_0237			0	5	0	4	0	0	0
Fjoh_0251		arginyl-tRNA synthetase	0	10	0	13	0	0	0
Fjoh_0260		heat shock protein 90	0	17	0	20	0	0	0
Fjoh_0394		50S ribosomal protein L2	0	5	0	2	0	0	0
Fjoh_0399		elongation factor G	0	27	0	44	0	0	0
Fjoh_0403		SusC-like protein	11	6	8	3	13	3	2
Fjoh_0405			4	7	2	5	4	6	5
Fjoh_0429		oligopeptidase A	14	17	9	18	10	12	18
Fjoh_0445		glycine dehydrogenase	15	21	16	24	15	16	18
Fjoh_0603			0	0	1	28	0	1	0
Fjoh_0609			2	0	2	1	4	3	6
Fjoh_0704		isoleucyl-tRNA synthetase	16	24	1	32	3	8	10
Fjoh_0822		glucosamine-fructose-6-phosphate	0	10	0	11	0	0	0

		aminotransferase							
Fjoh_0823			6	7	7	7	8	20	16
Fjoh_0857		methionyl-tRNA synthetase	0	4	0	8	0	0	0
Fjoh_0980/ SprD		motility protein	29	23	1	1	0	0	0
Fjoh_1012		glutaminyl-tRNA synthetase	0	6	0	10	0	0	0
Fjoh_1130		amidophosphoribosyltransferase	1	8	1	12	2	4	3
Fjoh_1248		aspartyl-tRNA synthetase	0	6	0	14	0	0	0
Fjoh_1255		dehydrogenase	0	5	0	3	0	0	0
Fjoh_1256		2-oxoglutarate dehydrogenase	0	23	0	28	0	0	0
Fjoh_1262		alpha,alpha-trehalase	11	22	16	17	12	22	16
Fjoh_1419		peptidase	7	15	0	9	1	5	2
Fjoh_1444		polynucleotide phosphorylase	13	17	3	24	9	7	6
Fjoh_1494		aspartate kinase	0	0	0	5	0	0	0
Fjoh_1504		sulfite reductase	4	5	0	5	1	3	3
Fjoh_1551		peptidase	0	4	3	2	3	8	7
Fjoh_1552		pyruvate dehydrogenase complex dihydrolipoamide acetyltransferase	0	17	0	15	0	0	0
Fjoh_1568			5	6	1	9	4	7	10
Fjoh_1608		peptidyl-dipeptidase Dcp	1	7	0	5	0	1	2
Fjoh_1651		malic enzyme	2	17	2	21	0	0	0
Fjoh_1722			3	11	1	7	1	4	3
Fjoh_1753		delta-1-pyrroline-5-carboxylate dehydrogenase	1	9	0	6	14	4	1
Fjoh_1764		phosphoglyceromutase	0	7	0	3	5	6	0
Fjoh_1842		chaperonin GroEL	4	98	0	63	14	1	4
Fjoh_1851		DNA topoisomerase I	0	8	0	6	1	0	0
Fjoh_1894		alanyl-tRNA synthetase	0	3	0	10	0	0	0
Fjoh_1908		urocanate hydratase	0	6	2	6	0	5	1
Fjoh_1919		lysyl-tRNA synthetase	11	18	8	13	5	8	9
Fjoh_1943		DNA-directed RNA polymerase subunit beta	0	12	0	21	0	0	1
Fjoh_1944		DNA-directed RNA	0	22	0	27	0	0	0



		polymerase subunit beta'							
		bifunctional aconitate hydratase/ 2-methylisocitrate dehydratase							
Fjoh_1951			2	22	0	23	0	0	1
Fjoh_1952		aconitate hydratase	2	8	3	14	2	4	5
Fjoh_2058			111	177	86	176	66	110	115
Fjoh_2059			48	118	39	120	24	73	79
Fjoh_2067			25	145	29	136	22	61	57
Fjoh_2068			1	110	5	91	10	23	22
Fjoh_2181		isocitrate dehydrogenase	42	55	32	63	29	45	51
Fjoh_2278		valyl-tRNA synthetase	0	2	0	6	0	0	0
Fjoh_2456	TIGR04183	pectate lyase	0	0	5	0	0	2	2
Fjoh_2556		oligopeptidase B	0	5	0	6	0	0	1
Fjoh_2584		DNA gyrase subunit A	0	14	0	14	0	0	0
Fjoh_2587			0	7	0	5	0	0	0
Fjoh_2631		chaperone DnaK	7	45	0	53	0	1	1
Fjoh_2703			1	0	1	0	2	0	5
Fjoh_2762		30S ribosomal protein S1	0	37	0	41	0	0	0
Fjoh_2916		ABC transporter ATP-binding protein	0	9	0	14	0	0	0
Fjoh_2968		phenylalanyl-tRNA synthetase subunit beta	2	5	3	8	1	3	1
Fjoh_3203	TIGR04183		1	0	5	0	0	6	5
Fjoh_3247			1	3	29	0	2	1	17
Fjoh_3422		ATPase	0	14	0	18	0	0	0
Fjoh_3475		NAD-dependent DNA ligase	0	6	0	4	0	1	3
Fjoh_3818			1	0	5	0	7	0	7
Fjoh_3881		SusD-like protein	2	3	3	5	2	15	10
Fjoh_4094		SusD-like protein	3	3	6	9	6	26	30
Fjoh_4512		transketolase domain-containing protein	7	16	0	22	1	6	5
Fjoh_4556		beta-N-acetylhexosaminidase	0	1	0	9	0	0	0
Fjoh_4557		glucosamine-6-phosphate deaminase	10	23	7	24	0	17	15
Fjoh_4720			0	5	1	2	1	2	0
Fjoh_4721	TIGR04183	endonuclease I	1	0	7	0	2	14	9

Fjoh_4723	TIGR04183	endonuclease I	2	1	17	0	7	21	24
Fjoh_4753		3-hydroxyacyl-CoA dehydrogenase	5	14	3	13	1	5	7
Fjoh_4756		acyl-CoA dehydrogenase	0	6	0	7	0	0	0
Fjoh_4812		glucosamine-6-phosphate deaminase	14	25	12	27	3	23	18
Fjoh_5004		preprotein translocase subunit SecA	0	2	0	7	0	0	0
Fjoh_5006			3	5	1	3	2	1	1
Fjoh_5040			1	4	1	5	1	2	2

<sup>a</sup> Proteins in cell-free culture fluid from wild type *F. johnsoniae* CJ1827,  $\Delta$ *gldNO* mutant CJ2090,  $\Delta$ *porV* mutant CJ2130,  $\Delta$ *porU* mutant CJ2116,  $\Delta$ *gldNO* complemented with pTB79,  $\Delta$ *porV* complemented with pSSK03, and  $\Delta$ *porU* complemented with pSSK04 were separated by SDS-PAGE, silver stained, and the regions shown in Figure 9 spanning approximately 60 to 240 kDa were cut from the gel and analyzed by LC-MS/MS. Total/unweighted spectrum counts corresponding to total number of spectra associated to a single protein and indicative of relative abundance of that protein are indicated for each of the 7 strains analyzed.

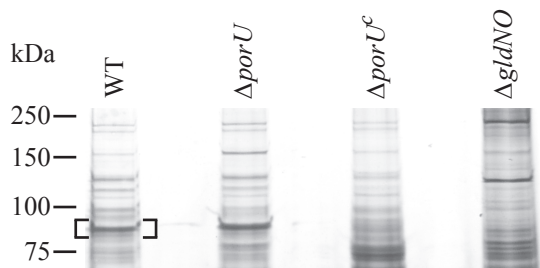
<sup>b</sup> CTD-type identified by BLASTP analysis.

<sup>c</sup> Predicted protein functions as listed on the Integrated Microbial Genomes website (<https://img.jgi.doe.gov>).

<sup>d</sup> The small number of spectra identified from wild type cells for these proteins made the prediction of secretion by the T9SS less certain. These proteins were included because no spectra were observed from  $\Delta$ *gldNO* or  $\Delta$ *porV* mutant cells and because complementation of the  $\Delta$ *gldNO* mutant resulted in 4 to 7 spectral hits for each protein.

<sup>e</sup> SprB is an abundant protein that is secreted by the T9SS but it remains attached to the cell surface rather than being released into the culture fluid (A Shrivastava et al. 2013. *J. Bacteriol.* 195: 3201-3212). This explains why it did not behave like a typical soluble secreted protein in this assay.

<sup>f</sup> ChiA has been shown to be secreted by the T9SS (see SS Kharade and MJ McBride. 2014. *J. Bacteriol.* 196:961-970). We do not know why ChiA did not behave more convincingly like a secreted protein in this analysis. The most intense band between 60 and 240 kDa in cell-free culture fluid of wild type cells (at approximately 90 kDa) was composed primarily of ChiA, and this band was not present in culture fluid of cells of the  $\Delta$ *gldNO* mutant suggesting that ChiA is secreted by the T9SS (see Figure S1 in the supplemental material for this paper).

**A****B**

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1 MKHYRLLFLLLFLLASAQPAHGKKVVGYAQWSIYARDFNVPKIDGSK
51 LTHLNYSFYGTTYDPAHEPENTKLIKCLDTYADFEHMEGGIPWDAFVKGNFY
101 DLMLKQKYPHLKILISVGGWTKGQDLSPIAASPVARAALAADMANFIV
151 YPFIDGFDIDWEYPLSGGTDGTEIVNGMFPVPPQKYSPDDNKNLVLLKAM
201 RQAMPNKLVTIAAGNNVRNVSKQYLGPNRAQYGMTEISTYCDYITYFG
251 YDFGGNWYDKTCYNAPLYASGNPNDPLYGATQSESLDELTNQYLVNIGFP
301 ANKLIMGLPFYGKFKFDNVAANSTNGLFVAAPRYIVPGCTNPQNPTGTWDG
351 SGACEKSGSIEICDLVGNPVTNSHAYLDPNTMMVTPSAASAGWVRYFDNT
401 TKVPYLYNSTLQKFISYEDKQSMDLKVQIKSRNLLAGMIWELSQDTRGS
451 IPNSLLNQVDTSFGSVVPGTVSISGSVKNGSALVTDVTVELRNASNAVIQ
501 TVVSANGNFAFNLLTSGQNYSLTALKATYTFFTPVTLVNVTVNQTAVVING
551 TQPTYTVSGTVLDGSTPVSGVTVTAVSGSTTLTAVSNASGVYSIAGLTAG
601 LNFTVTAAKSGFSYAPASTVYNAIDSNKTLNFTQGAPVVNYTVSGTVLNS
651 TTPVSGVTVTASFTGGSYAAVTNASGTYSLSLSPGGNYTVTAALTGQTFT
701 PASTVYSNLNANKTLNFTQDVVSTSKISGTVKNGTNPVAGAKVELVLPW
751 TDNTHNWSVIAATDAQGYKYSFDNSVVDGYTQVLSLKLNSWQNGEVAYYP
801 NNLANFAVPANPTVYNFNTSSTAKSALAAAANLISGTVKNGTTPVAGAKV
851 EIVLPWTDNTHNWSVLATTDASGNYSFDNSVVAGYTQILSLKLNGWENG
901 DVTYYPNNLANFAVPTTPTIYFNRQAVVATKPVVITAPTASAIAINLG
951 SAINFVASVGLSAVDATTISSVVFLDGGQSLSTANSSGTYTAAWTPAANQ
1001 FLSHTLTVTATASNGTTDSKTYSFTLTCSGANCPNALPVITWNSPSNTT
1051 VYQNTFQVVPISVTAVSDGTVSGVTITINGGTFNMTAGTNNTYTYNFTP
1101 SAYQDYPVVIKATDNKSGVTTLNNTIKIATVSTNRFIPLPSKIILGYAS
1151 WENAGAPFLYFSQMVGSKFNVVDYSFVETVNRDGYTPLLTTNDTRYLTNG
1201 VFNKQLLKNDIKSLRDSGVPVIVSIGGQNGHVLDNVTQKNIFVNLKAI
1251 IDEYQFDGVDIDFEGGSMNFNAGLRDISYAGISAYPRLKNVDAFKELK
1301 AYYGPGFLLTAAPETQYVQGGYTTYTDTFGSFLPIIQNLRNELDLLAVQL
1351 YNTGGENGLDGQYYGTAKKSNMVTALTDMVIKGYNIASTGMRFDGLPASK
1401 VLIALPACPSAAGSGYLTPTEGINAMHYLRTGTFSGRTYTMQPGGPYPS
1451 LRGLMTWSVNWDASSCGNSSELSKAYAYFASQTAAKTLVLDDISAKSNA
1501 TIAYFKNNALSVTNENEDIAQVDVFNVLGQNLVSHRNVQNNKEVLLHNQS
1551 FSSKQLFLVVVTDKAGNKSKFKVMNFLN

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Figure S1. The approximately 90 kDa protein present in culture fluid of wild-type cells but not in culture fluid of the *ΔgldNO* mutant corresponds to the N-terminal portion of ChiA. A) Cell-free spent medium from wild-type cells (WT) and from cells of the *ΔporU* mutant, *ΔporU* mutant complemented with pSSK04, and *ΔgldNO* mutant were separated by SDS-PAGE and proteins were detected by silver staining. The approximately 90 kDa band that was present in the cell-free culture fluid of wild-type cells (bracketed) but was not present in culture fluid of the *ΔgldNO* mutant was cut from the gel and subjected to LC-MS/MS analysis. B) The primary amino acid sequence of ChiA is shown, with the regions detected by LC-MS/MS highlighted in red. 469 spectral matches were detected to ChiA. All of these except for 3 fell between amino acids 26 and 864, which corresponds to the amino-proximal region of the protein after removal of the signal peptide. ChiA has previously been shown to be processed into N-terminal and C-terminal fragments, each of which has a chitinase catalytic domain (SS Kharade and MJ McBride. 2014. *J. Bacteriol.* **196**:961-970).