SUPPLEMENTAL MATERIAL

Supplemental Materials and Methods

In separate file.

Supplementary Table 1

List of strains used and origin (in separate file)

Supplementary Table 2

List of primers used (in separate file)

Supplementary Figure Legends

FIG S1 Pairwise identities among F-type pyocin proteins encoded at the 3'-end of the operon. All against all pairwise percent identities for the indicated proteins are shown. Based on the comparison of homologous proteins encoded within this region, F-type pyocins were classified into 11 distinct groups, of which 6 groups were experimentally tested. The F-pyocin groups of the proteins that are being compared are indicated at the sides and top of each table. Numbers that are shaded denote groups that share Specificity Modules. The PyoF10 comparisons include only the C-terminal 60 amino acids. PyoF13 comparisons include only the last 210 residues of these proteins. The F7 group does not encode PyoF14, so these boxes are left blank. "<20" denotes sequences that could not be well aligned in a pairwise alignment. F21 and F22 refer to the duplicated PyoF13, PyoF14, and PyoF15 proteins encoded in the F2 group.

FIG S2 Protein sequence alignments of PyoF11 and PyoF12 from each F-type pyocin group. (a) An alignment of PyoF11 homologs is shown from the 11 F-type pyocin groups and selected phages. (b) An alignment of PyoF11 homologs is shown from the 11 F-type pyocin groups and selected phages. The phage proteins are from *Burkholderia* phages KS9 (NC_013055) and BcepGomr (NC_009447); *P. aeruginosa* phages LIT1 (NC_013692) and LUZ7 (NC_013691); and *E. coli* phages T1 (NC_005833) and N15 (NC_001901).

FIG S3 Protein sequence alignments of PyoF13 from each F-type pyocin group. (a) An alignment of the N-terminal 140 amino acids of PyoF13 homologs from the 11 F-type pyocin groups is shown. (b) An alignment of the C-terminal 210 amino acids of PyoF13 homologs from the 11 F-type pyocin groups is shown.

FIG S4 Protein sequence alignments of the tail fiber proteins (PyoR6) from each R-type pyocin group. (a) An alignment of the N-terminal 450 amino acids of the PyoR6 homologs is shown. (b) An alignment of the C-terminal 250 amino acids PyoR6 homologs is shown. (c) The pairwise sequence identities of the PyoR6 N-terminal and C-terminal regions are shown. The locus tags for the proteins shown are R1 (PLES_06171), R2 (PA14_08050), and R5 (PA0620).

FIG S5 The bactericidal activity of all F-type pyocin lysates tested on all of the strains used in this study. All lysates were spotted both on plates containing proteinase K and plates without proteinase K. Conclusions about pyocin types within each lysate were derived from examination of both plates. In lysates containing R- or F-type pyocin activity, S-type pyocin activity may not have been detectable in some cases because R- and F-type activity tended to be stronger and could mask the S-type activity. ”NT” denotes a non-typeable serotype.

Supplemenatary Table 1. Strains used.

Strain	Original Designation	Place of Isolation	Source
PA14	PA14		Human
PAO1	PA01	Australia	Human
S1	RYC97083283	Spain	Human
S2	PA100420	Canada	Human
S3	CF040	USA	Human
S4	EnvJH	Canada	Environment
S5	PA131533	Canada	Human
S6	RR1	Spain	Environment
S7	PA191517	Canada	Human
S8	PA4944	Canada	Human
S9	Env24 DJ	Canada	Environment
S10	CECT116	Spain	Environment
S11	Env23 DJ	Canada	Environment
S12	EnvKY2	USA	Environment
S13	ATCC15528	USA	Environment
S14	EnvBC13	Canada	Environment
S15	T4347	Trinidad	Human
S16	Env110 BP	Canada	Environment
S17	Env34 DJ	Canada	Environment
S18	2709	Belgium	Human
S19	EnvBC15	Canada	Environment
S20	EnvBC10	Canada	Environment
S21	ATCC15524	USA	Environment
S22	EnvCIN1	USA	Environment
S23	SA2	South Africa	Environment
S24	Env63 BP	Canada	Environment
S25	Env25 DJ	Canada	Environment
S26	CF049	Canada	Human
S27	T4826	Trinidad	Human
S28	PML14	N/A	N/A

Supplementary Table S2. Primers used.

Primer name	Primer sequence
PA14*pyoF11*-For	CCCGGGCCATGGCCGAAGTGCGCCAGGAATGG
PA14*pyoF11*-Rev	CCCGGGAAGCTTTCAATACACGCCATAAATCAC
PA14*pyoF11*gib-For	TATACATACCCATGGGATCTGATAAATGAACAAGACCATCACGGGC
PA14*pyoF12*gib-Rev	ACGTTGTAAAACGACGGCCAGTGCCTTAATAACCAGTCACATCTACCA CCAGATATCC
20T-gib-For	GGCACTGGCCGTCG
20T-gib-Rev	TTATCAGATCCCATGGGTATGTATATCTCCTTCT
PA14*pyoF12*-For	CCATGGCCTTGATGATTGGGTTTAAGG
PA14*pyoF12*-Rev	CTGCAGTTAATAACCAGTCACATCTAC
PA14*pyoF13*-For	CCCGGGGGATCCATGACTTGGTATTCCAGTGGC
PA14*pyoF13*-Rev	CCCGGGAAGCTTTCAGTACCACCTCCCCACAGC
PA14*pyoF14*-For	CCCGGGCCATGGCCCGTATCGAACTCAGTCC
PA14*pyoF14*-Rev	CCCGGGAAGCTTTCACTTGGGCAACTCCACG
PA14*pyoF15*-For	CCCGGGCCATGGCCGTGATCGATTGGAAAATG
PA14*pyoF15*-Rev	CCCGGGAAGCTTTCAGATGCGCGGGAGCG
PA14*pyoF11*-delR	CAGACCGACCGTCACGACAGCAATCC
PA14*pyoF11*-delF	GGAGATGTAATTTCATGGGACTTTCCCGGG
PA14*pyoF11*rec-F	CCCGGGGAATTCGATACCGTCAGCGCGCGCACCGATA
PA14*pyoF11*rec-R	CCCGGGAAGCTTATATCTTGAGTCATACACAACACCGCC
PA14CR*pyoF11*-del-F	CATGGGTTCACTGCCGTGTAGGCAGCTAAGAAAGATTTTTCGTCGGGT TCTGGGTGGGCTTGCATCGTTCACTGCCGTGTAGGCAGCTAAGAAAA
PA14CR*pyoF11*-del-R	AGCTTTTTCTTAGCTGCCTACACGGCAGTGAACGATGCAAGCCCACCC AGAACCCGACGAAAAATCTTTCTTAGCTGCCTACACGGCAGTGAACC
PA14*pyoF13*-rec-F	CCCGGGGAATTCAACTCTGGCCGTTGATGG
PA14*pyoF13*rec-R	CCCGGGCTGCAGGTCCCCACGATATTGTCT
PA14*pyoF13*sdm-F	ATTCCAGTGGCACCTGAGCGGTGACCGCAAAT
PA14*pyoF13*sdm-R	ATTTGCGGTCACCGCTCAGGTGCCACTGGAAT
PA14CR*pyoF13*-sdm-F	CATGGGTTCACTGCCGTGTAGGCAGCTAAGAAAGTAGCGGTGACCGCA AATAGCCCGACCGTTACGTTCACTGCCGTGTAGGCAGCTAAGAAAA
PA14CR*pyoF13*-sdm-R	AGCTTTTTCTTAGCTGCCTACACGGCAGTGAACGTAACGGTCGGGCTA TTTGCGGTCACCGCTACTTTCTTAGCTGCCTACACGGCAGTGAACC
SS633	TTGTATTTCCAGGGCTCCTCTGGTTCAACAGTTCCAG
SS635	CGGTACCCACTCACCAGTCCTTAAACCCAATCATCAA
SS636	GAACCGAACAGGCTTATGTCAATTTATCTGGTGGTAGATGTGAC
SS634	CAAGCTTCGTCATCAAGCGGGTACTGGCGTCGAATAC
SS639	TCCTCTGGTTCAACAGTTCCAGC
SS640	AGCGGGTACTGGCGTCGAATAC

SS545	**ACTGGTGAGTGGGTACCG**
SS546	**AATTGACATAAGCCTGTTCGGTTC**
PA14*pyoR1*rec-F	CCCGGGCCATGGCCCCTGAACAGGCTGTTACG
PA14*pyoR2*rec-R	CCCGGGAAGCTTTCAATGGGTGTGGTGGTTGCT
PA14*pyoR2*sdm-F	GCGGAGCATGACCGCTAGCTCGCCGCGATGAT
PA14*pyoR2*sdm-R	ATCATCGCGGCGAGCTAGCGGTCATGCTCCGC
PA14CR*pyoR2*-del-F	CATGGGTTCACTGCCGTGTAGGCAGCTAAGAAAGCATGCTCGCCGCGA TGATCCTGCCCTGCGTGGGTTCACTGCCGTGTAGGCAGCTAAGAAAA
PA14CR*pyoR2*-del-R	AGCTTTTTCTTAGCTGCCTACACGGCAGTGAACCCACGCAGGGCAGGA TCATCGCGGCGAGCATGCTTTCTTAGCTGCCTACACGGCAGTGAACC
PA14*pyoF10*-rec-F	CCATGGAACAACACCCAGCTCTCGGCGG
PA14*pyoF10*-rec-R	AAGCTTCTCCACACTGCTGGCGTAGACCG
PA14*pyoF10*-del-F	CGCGTGCTGACCGTGACCGCC
PA14*pyoF10*-del-R	GGTCCAGGCCGACTTGAACGAGCC
PA14CR*pyoF10*-del-F	CATGGGTTCACTGCCGTGTAGGCAGCTAAGAAAGCATCGCCCAGTACT GCGACCAGTCGGTGCCCGGTTCACTGCCGTGTAGGCAGCTAAGAAAA
PA14CR*pyoF10*-del-R	AGCTTTTTCTTAGCTGCCTACACGGCAGTGAACCGGGCACCGACTGGT CGCAGTACTGGGCGATGCTTTCTTAGCTGCCTACACGGCAGTGAACC
SS246	ATGTCCATCCTGACTCAAGGTAC
SS247	TCAAGCCGACTTCGGCGTCCAC

PyoF10 (C-terminus)

	F1	F2	F4	F5	F6	F7	F8	F9	F10	F11
F2	42									
F4	39	43								
F5	31	28	33							
F6	36	28	60	32						
F7	37	26	53	34	52					
F8	93	42	41	31	36	35				
F9	26	26	31	47	41	38	26			
F10	43	43	97	36	60	57	44	31		
F11	28	30	36	36	32	33	29	33	36	
F12	28	30	36	36	32	33	29	33	36	100

PyoF11

	F1	F2	F4	F5	F6	F7	F8	F9	F10	F11
F2	30									
F4	20	22								
F5	19	22	28							
F6	24	<20	35	30						
F7	24	19	22	<20	16					
F8	93	32	22	18	21	23				
F9	26	24	24	23	22	22	26			
F10	22	21	93	27	36	22	22	23		
F11	<20	<20	35	28	30	<20	21	<20	35	
F12	<20	<20	35	28	30	nd	21	<20	35	99

PyoF12

	F1	F2	F4	F5	F6	F7	F8	F9	F10	F11
F2	25									
F4	<20	<20								
F5	<20	<20	19							
F6	20	<20	25	<20						
F7	28	24	22	<20	<20					
F8	73	26	<20	<20	<20	26				
F9	21	20	nd	20	<20	23	23			
F10	<20	<20	96	21	25	<20	<20	<20		
F11	<20	23	21	24	23	<20	<20	<20	23	
F12	<20	23	21	24	23	<20	<20	<20	23	99

PyoF13 (C-terminus)

	F1	$F2_2$	F4	F5	F6	F7	F8	F9	F10	F11	F12
$F2_2$	27										
F4	27	100									
F5	28	100	100								
F6	100	27	27	27							
F7	<20	<20	<20	<20	<20						
F8	47	32	32	33	47	<20					
F9	41	33	33	33	41	<20	76				
F10	51	30	30	31	51	19	51	43			
F11	28	100	100	100	28	<20	33	33	30		
F12	52	30	30	31	52	19	52	44	99	31	
$F2_1$	27	23	23	23	26	<20	25	24	34	23	34

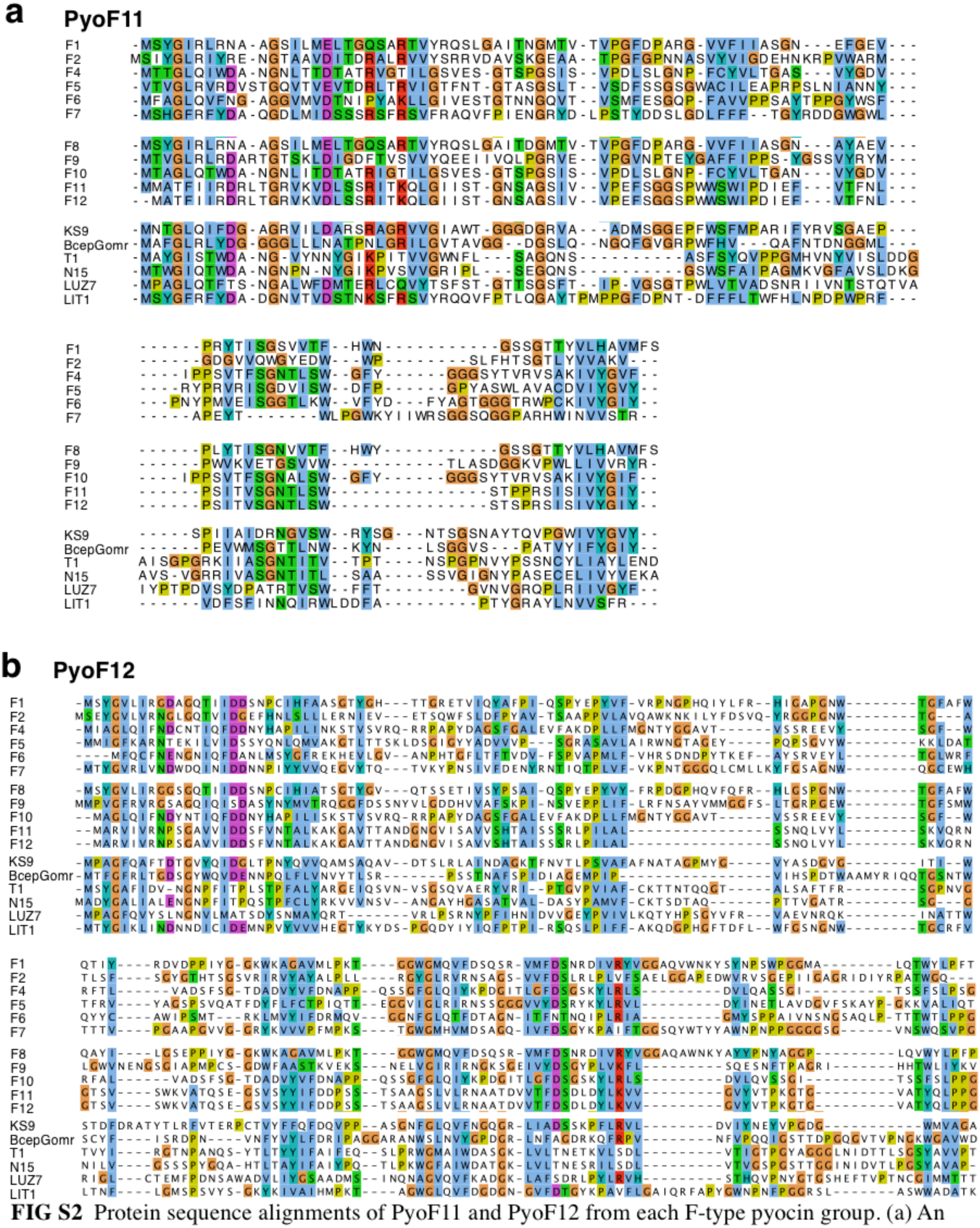
PyoF14

	F1	$F2_2$	F4	F5	F6	F7	F8	F9	F10	F11	F12
$F2_2$	31										
F4	31	100									
F5	31	100	100								
F6	98	33	33	33							
F7											
F8	38	46	46	46	38						
F9	37	43	43	43	37		84				
F10	38	47	47	47	38		90	82			
F11	31	100	100	100	31		46	43	47		
F12	37	47	47	47	37		88	81	98	47	
$F2_1$	48	28	28	28	48		23	27	25	28	26

PyoF15

	F1	$F2_2$	F4	F5	F6	F7	F8	F9	F10	F11	F12
$F2_2$	68										
F4	68	98									
F5	66	99	96								
F6	99	68	68	66							
F7	53	41	41	41	55						
F8	89	68	68	66	91	46					
F9	76	61	61	59	77	49	84				
F10	91	68	68	66	92	49	99	84			
F11	66	99	96	100	66	41	66	59	66		
F12	89	68	68	66	91	46	100	84	99	66	
$F2_1$	60	52	52	52	60	45	58	55	63	52	58

FIG S1 Pairwise identities among F-type pyocin proteins encoded at the 3'-end of the operon. All against all pairwise percent identities for the indicated proteins are shown. Based on the comparison of homologous proteins encoded within this region, F-type pyocins were classified into 11 distinct groups, of which 6 groups were experimentally tested (F1 through F7). The F-pyocin groups of the proteins that are being compared are indicated at the sides and top of each table. Numbers that are shaded denote groups that share Specificity Modules. The PyoF10 comparisons include only the C-terminal 60 amino acids. PyoF13 comparisons include only the last 210 residues of these proteins. The F7 group does not encode PyoF14, so these boxes are left blank. "<20" denotes sequences that could not be well aligned in a pairwise alignment. F21 and F22 refer to the duplicated PyoF13, PyoF14, and PyoF15 proteins encoded in the F2 group.



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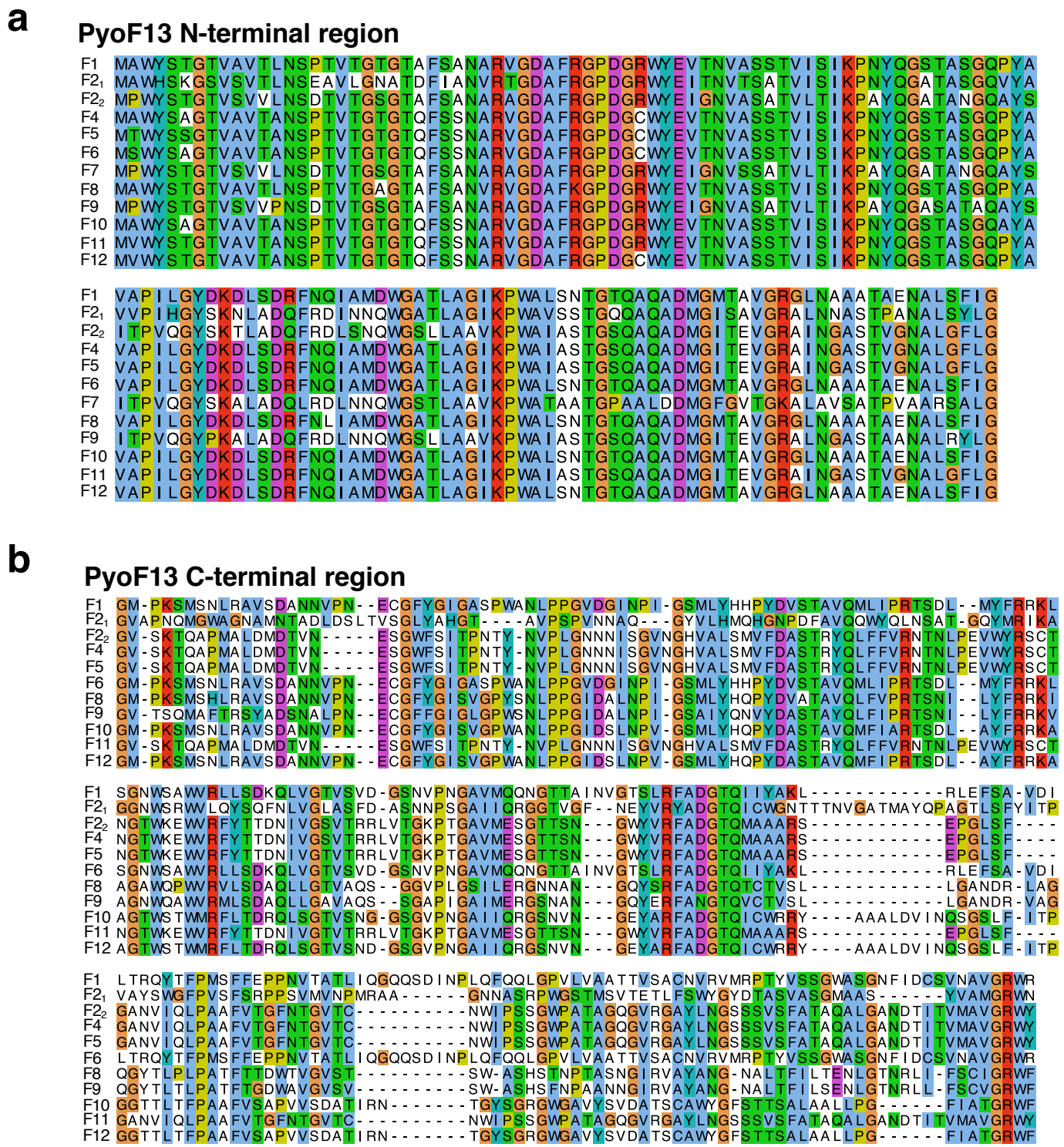

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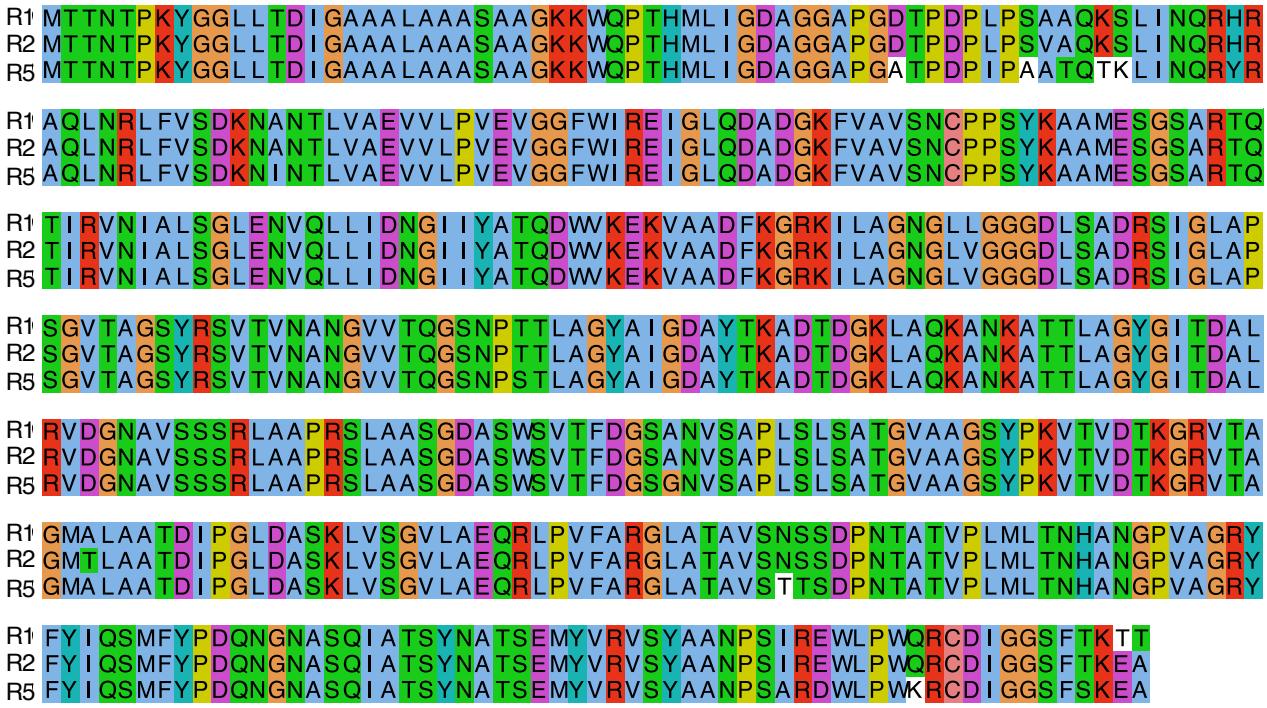
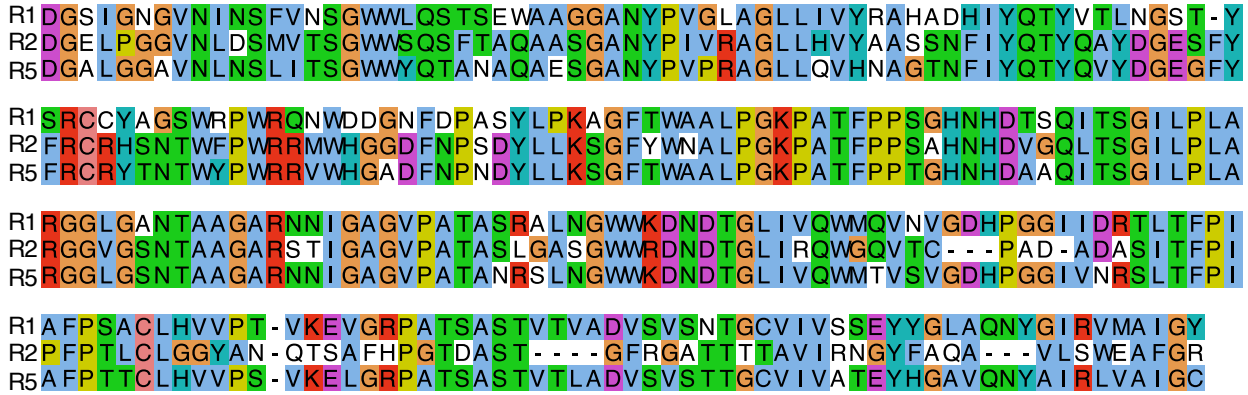
a



b



c

N-terminal region

Pairwise % identities

	R2	R5
R1	99	96
R2		96

C-terminal region

Pairwise % identities

	R2	R5
R1	53	70
R2		61

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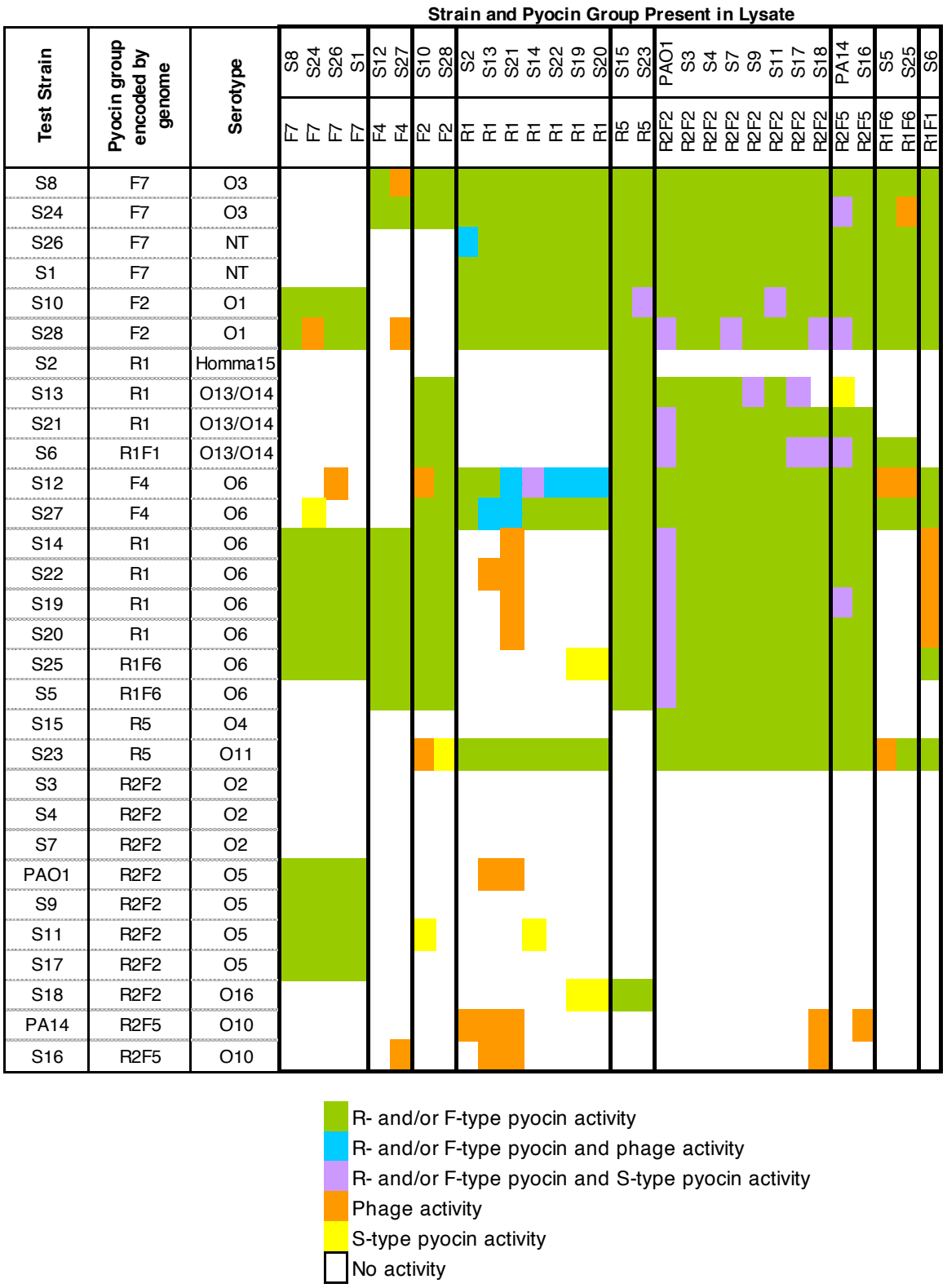

FIG S5 The bactericidal activity of all F-type pyocin lysates tested on all of the strains used in this study. All lysates were spotted both on plates containing proteinase K and plates without proteinase K. Conclusions about pyocin types within each lysate were derived from examination of both plates. In lysates containing R- or F-type pyocin activity, S-type pyocin activity may not have been detectable in some cases because R- and F-type activity tended to be stronger and could mask the S-type activity. ”NT” denotes a non-typeable serotype.