

**Supplemental Table 1.** Oligonucleotides used in this study.

Oligonucleotide	Description	Sequence
<b>qPCR oligos:</b>		
311	rot-6xHis-3'-RXho	5'-CCCTCGAG-TTAGTGATGGTGATGGTGATG-CACAGCAATAATTGCGTTAAC
313	rot5'F-NdeI	5'-CCCC-CATATG-AAAAAAAGTAAATAACGACACTG
491	paur-F-PstI	5'-CCC-CTGCAG-TTGATTATGATTATTTAAATAGTTATC
492	paur-R-KpnI	5'-CCC-GGTACC-CTTATAAATAAAATTTAATTAAATTTTC
493	pssp-F-PstI	5'-CCC-CTGCAG-CATTCATATTAAATCTACCTTGGC
494	pssp-R-KpnI	5'-CCC-GGTACC-CCTCCAAAAAAATTATTTACAAGTTA
495	pstaphopain-F-PstI	5'-CCC-CTGCAG-GTGTGGCATTAAATATAAGACAT
496	pstaphopain-R-KpnI	5'-CCC-GGTACC-CCTTATTAAATTATAAAATAATTATTTA
497	pspl-F-PstI	5'-CCC-GGTACC-GGGAACTAATCGCTAATAATGTT
498	pspl-R-KpnI	5'-CCC-GGTACC-CAAATTATTTATAGTTAATGTATCT
499	paur-R-BIO	5'-CTTATAAATAAAATTTAATTAAATTTTC
500	pssp-R-BIO	5'-CCTCCAAAAAAATTATTTACAAGTTA
501	pstaphopain-R-BIO	5'-CCTTATTAAATTATAAAATAATTATTTA
502	pspl-R-BIO	5'-CAAATTATTTATAGTTAATGTATCT
87	LukAB intra 5'	5'-CCCCGAATTCAAAGAAGGATAATATTGAAAGG
372	LukAB intra 3'	5'-AGTATCACCATCAAGATTCTTC
278	16S P1-RT	5'-TGAGATGTTGGGTTAAGTCCCGCA
279	16S P2-RT	5'-CGGTTTCGCTGCCCTTGTATTGT
879	aureolysin-F	5'-GGCAGCATTAAACCTTGTGA
880	aureolysin-R	5'-TTAATGCTTGACCGCATC
881	staphopain-F	5'-CTATTGCAAACGCTGAGAGC
882	staphopain-R	5'-ACGTACGTCAGTAGGAACACTCTT
883	ssp V8 protease-F	5'-CAAGCAAACAGCAAACACCT

884	ssp V8 protease-R	5'-TGGTGACGATCGTTATTGG
887	splF-F	5'-AGTGGTGTACATGGATGGG
888	splF-R	5'-TCATCACCGACTTCATGTG
789	rot-F	5'-AAGAGCGTCCTGTTGACGAT
790	rot-R	5'-TTTGCATTGCTGTTGCTCTA
<b>Cloning Oligos</b>		
JMM032	pOS1-MCS	5'-TAT-GTA-AGG-TAC-CAC-GCG-TGA-GCT-CTC-TAG-AC
JMM033	pOS1-MCS	5'-TCG-AGT-CTA-GAG-AGC-TCA-CGC-GTG-GTA-CCT-TAC-A
VJT423	rot mutant	5'-GGGGACAAGTTGTACAAAAAAAGCAGGCTTACACCGATGGTAAAACAATACG
VJT424	rot mutant	5'-TCCCCCCCAGGTGTAAAGTTAACATGCTAAAAAG
VJT425	rot mutant	5'-TCCCCCCCAGGTGTAAACATCTCCC
VJT426	rot mutant	5'-GGGGACCACCTTGTACAAGAAAGCTGGGTTCATGACAGTATTGAATGATG

Genes negatively regulated by Rot				
			Fold higher in <i>ro</i>	
Locus	gene name	description	WT (mid log)	WT (late log)
SACOL0128		phosphonate ABC transporter, phosphonate-binding protein	2.8	
SACOL0155		cation efflux family protein		2.6
SACOL0156		hypothetical protein		2.8
SACOL0163		drug transporter, putative	2.1	
SACOL0189		hypothetical protein	2.3	
SACOL0198		conserved hypothetical protein		3.6
SACOL0222	ldh	L-lactate dehydrogenase		2.4
SACOL0253	rbsk	ribokinase		2.2
SACOL0254		ribose transport protein		2.6
SACOL0270		staphyloxanthin biosynthesis protein, putative	2.4	
SACOL0271		conserved hypothetical protein		2.2
SACOL0285		conserved hypothetical protein	2.5	
SACOL0285		conserved hypothetical protein	2.6	5.7
SA0352		30S ribosomal protein S6		2.9
SACOL0357	dut	prophage L54a, deoxyuridine 5-triphosphate nucleotidohydrolase		2.9
SACOL0454		sodium:dicarboxylate symporter family protein		2.4
SACOL0492		hypothetical protein		2.5
SACOL0645		conserved hypothetical protein	2.1	
SACOL0858	empbp	secretory extracellular matrix and plasma binding protein		2.2
SACOL0865		hypothetical protein	2.2	
SACOL0869		phosphoglycerate mutase family protein		2.5
SACOL0892		pathogenicity island protein, authentic frameshift		8.7
SACOL0985		surface protein, putative		6.0
SACOL1041		hypothetical protein	3.2	
SACOL1349		conserved hypothetical protein	5.4	2.1
SACOL1387		conserved hypothetical protein	2.8	2.3
SACOL1415		peptide ABC transporter, ATP-binding protein	5.8	
SACOL1416		peptide ABC transporter, permease protein, putative	4.2	
SACOL1417		peptide ABC transporter, permease protein	5.5	
SACOL1476		amino acid permease		2.5
SACOL1481		conserved hypothetical protein	3.6	
SACOL1532		hypothetical protein	7.0	
SACOL1532		hypothetical protein	8.4	3.5
SACOL1533		hypothetical protein	3.5	
SACOL1769	rpsD	ribosomal protein S4		3.7
SA1771		conserved hypothetical protein		2.4
SA1773		conserved hypothetical protein		2.6

SA1774		conserved hypothetical protein		2.3
SA1775	clp	conserved putative Clp protease		2.2
SA1780		conserved hypothetical protein		2.5
SA1783		conserved hypothetical protein		2.8
SA1790		conserved hypothetical protein		3.7
SA1792		single-strand DNA-binding protein		2.8
SA1795		conserved hypothetical protein		2.3
SA1799		conserved hypothetical protein		3.2
SA1803		conserved hypothetical		2.4
SA1804		conserved DNA binding protein- putative transcriptional regulator		3.5
SACOL1880	lukD	leukocidin D		3.5
SACOL1865	splE	serine protease SplE, putative	43.7	
SACOL1866	splD	serine protease SplD, putative	28.5	
SACOL1870		hypothetical protein	27.2	
SACOL1971		hypothetical protein	3.7	2.1
SACOL1993		conserved hypothetical protein		2.1
SACOL1995		conserved hypothetical protein		2.8
SACOL2004	lukA	leukocidin A		2.4
SACOL2006	lukB	leukocidin B	2.3	
SACOL2220	rplO	ribosomal protein L15		2.6
SACOL2221	rpmD	ribosomal protein L30p/L7e		2.4
SACOL2222	rpsE	ribosomal protein S5		2.2
SACOL2223	rplR	ribosomal protein L18		2.2
SACOL2224	rplF	ribosomal protein L6		2.4
SACOL2225	rpsH	ribosomal protein S8		2.2
SACOL2226	rpsN2	ribosomal protein S14		2.6
SACOL2227	rplE	ribosomal protein L5		2.2
SACOL2228	rplX	ribosomal protein L24		2.3
SACOL2231	rpmC	ribosomal protein L29		2.1
SACOL2279		transporter, putative	4.2	
SACOL2353	tcaR	transcriptional regulator TcaR		8.6
SACOL2361		hypothetical protein		3.6
SACOL2451		amino acid ABC transporter, amino acid-binding protein	2.0	
SACOL2479		conserved hypothetical protein		2.2
SACOL2552		PTS system, IIABC components		4.1
SACOL2559		hypothetical protein	2.1	
SACOL2601		conserved hypothetical protein	2.4	4.1
SACOL2663		PTS system, fructose-specific IIABC components		5.8
SACOL2664	manA	mannose-6-phosphate isomerase		6.2
SACOL2669		conserved hypothetical protein		2.1
SACOL0492		hypothetical protein	5.6	

SA0882		conserved hypothetical protein, similar to competence transcription factor	3.0	
SA0901	sspA	serine protease; V8 protease; glutamyl endopeptidase	29.9	15.0
SACOL2007		peptidase, M20/M25/M40 family, authentic frameshift	2.7	
SAS018		conserved hypothetical protein	2.5	
SACOL0136	cap5A	capsular polysaccharide biosynthesis protein		4.3
SACOL0137	cap5B	capsular polysaccharide biosynthesis protein		3.5
SACOL0138	cap5C	capsular polysaccharide biosynthesis protein	6.4	2.8
SACOL0139	cap5D	capsular polysaccharide biosynthesis protein	5.8	
SACOL0140	cap5E	capsular polysaccharide biosynthesis protein	4.6	2.4
SACOL0141	cap5F	capsular polysaccharide biosynthesis protein	3.6	2.3
SACOL0142	cap5G	UDP-N-acetylglucosamine 2-epimerase		3.4
SACOL0143	cap5H	capsular polysaccharide biosynthesis protein		5.7
SACOL0144	cap5I	capsular polysaccharide biosynthesis protein	5.2	5.4
SACOL0145	cap5J	capsular polysaccharide biosynthesis protein		7.8
SACOL0146	cap5K	capsular polysaccharide biosynthesis protein	3.1	6.5
SACOL0147	cap5L	capsular polysaccharide biosynthesis protein		6.8
SACOL0148	cap5M	capsular polysaccharide biosynthesis galactosyltransferase		5.5
SACOL0149	cap5N	capsular polysaccharide biosynthesis protein	2.4	5.3
SACOL0150	cap5O	capsular polysaccharide biosynthesis protein		3.9
SACOL0151	cap5P	UDP-N-acetylglucosamine 2-epimerase		4.6
SACOL2003	hlb	phospholipase C	8.4	
SACOL2422	hlgB	gamma hemolysin, component B	5.2	
SACOL2421	hlgC	gamma hemolysin, component C	5.3	
SACOL1173	hly	alpha-hemolysin precursor	2.8	
SACOL2068	kdpA	potassium-transporting ATPase, A subunit	8.3	
SACOL2067	kdpB	potassium-transporting P-type ATPase, B subunit	4.4	
SACOL2066	kdpC	potassium-transporting ATPase, C subunit	2.9	
SACOL2070	kdpD	sensor histidine kinase KdpD	7.8	
SACOL2071	kdpE	DNA-binding response regulator KdpE	6.4	
SACOL0248	IrgB	IrgB protein	2.5	
SACOL1881	lukE	leukocidin E	10.3	
SACOL2002	map	map protein, authentic frameshift	2.3	
SACOL0078	plc	1-phosphatidylinositol phosphodiesterase	10.9	
SACOL1869	splA	serine protease SplA	74.9	
SACOL1868	splB	serine protease SplB	42.0	
SACOL1867	splC	serine protease SplC	64.4	
SACOL1056	sspB1	cysteine protease precursor SspB1	30.5	17.4
SACOL1970	sspB2	cysteine protease precursor SspB2	6.0	
SACOL1055	sspC	sspC protein	24.9	19.4
SACOL0164		gramicidin S synthetase 2 related protein	2.3	
SACOL1481		conserved hypothetical protein	3.1	

<i>t</i> strain in comparison to	
Complement (mid log)	Complement (late log)
3.8	
	3.7
	3.1
3.9	
3.4	
	5.8
	5.3
	2.6
	2.8
7.7	
	4.3
4.8	
4.5	16.1
	3.3
	9.9
	4.7
	3.8
2.9	
	3.5
4.0	
	2.5
	9.7
	5.9
18.0	
7.5	2.4
4.5	5.8
8.1	
6.1	
8.1	
	5.9
4.7	
7.2	
6.9	2.6
4.4	
	8.6
	4.4
	4.8

	5.8
	3.9
	4.5
	7.1
	8.9
	8.2
	5.4
	7.8
	8.4
	7.3
	5.5
47.5	
27.9	
59.3	
8.1	16.1
2.7	
2.6	
11.8	
9.4	
	3.4
	4.8
	4.1
	4.8
	4.6
	4.3
	5.6
	4.4
	4.8
	4.5
3.4	
	13.1
	3.3
2.2	
	2.1
	6.5
2.8	
4.9	2.7
	14.7
	15.4
	2.4
6.8	

7.2	
44.8	23.3
6.3	
5.9	
	18.7
	9.3
9.8	5.9
6.0	
5.2	6.8
7.9	12.0
	10.7
	25.2
5.0	23.8
	18.7
2.8	18.4
	16.9
	16.9
2.2	15.3
	12.8
	11.6
84.9	
11.0	
11.9	
9.7	
9.5	
4.6	
3.4	
21.6	
17.9	
4.6	
16.2	
6.1	
41.0	
90.5	
37.6	
60.6	
47.6	38.2
20.7	
33.4	39.3
4.2	
4.1	