

## **Supplementary information**

### **Rgg-Shp regulators are important for pneumococcal colonization and invasion through their effect on mannose utilization and capsule synthesis**

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**SFile 1:**

>SPD\_0144 WP\_000570730.1

MIEKMELGEFYKELRLARKLKQTDVACEGLTASQLSKFELGQSMLSADKLILAIQGIN  
VTFDEFGHKLNNYQESPHMRIGRKVVNRFAHQDIAALEQLLEEVDQEQMAQTYRR  
LNAIVIK

DAIHSLNKSYPPLAEEDSEFLTYYLYAIESWTWFELYLFCNTMPFLSNQDLIFLSTSL  
EKSKEFKELVHNRLYMKQGILLNLSSELMERKLFSYIPIFEAELERMLRPYDVFEKVS  
WQFLK KMSVFLQTKGSNQKEIERFIQSLQVLENPQLTSLFELRFQQYKELID

>SPD\_0939 WP\_000837499.1

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H

TMDSNIIPSDEELLHLTDYLFKIEKWGYEILLGNCVVRTINYNSYFLLTKEMLNYYIS  
SLNKTNKQIVSQLAINCFILSIDKEEFSNCSYLISKIKTLLDNELNFYEQTVFLYATGYF  
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>SPD\_0999 WP\_000400550

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NWILLQ

GLICQRDASYDMKQDDLDKVADYLFKTEEWMTMYELILFGNLYSFYDVDYVTRIGRE  
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>SPD\_1518 WP\_001267153

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YRHTK

IVTELRTKGFKNNHVLKDLYEIWDYLEPMDTWYISDLKLLGTILFFFPSENPLIDRI  
MKTIEKYKYFRETKAFLSSFLANLSTVYFQHHLFKECETITLQLLVLAEEELKIYDILGFS  
QVRLGILQHNSDLIDKGITLLRLTKEEALVKILEKEINDFSNL

>SPD\_1952 WP\_000434947.1

MEQIGKVFRQLRESRNISLRQATGGQFSPSMLSRFETGQSELLVEKFLFALENISAS  
VEEILFLARGFYDSTDSELRKEITDVLEPKNIAPLEDLYRREYQKHAHSYNKQKHILN

AIHKSYMKSIDERVDLTAEKGVLHDYLFSTEIWGIYELNLFVSSPFLSVSLFTRYV  
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>C1 WP\_000427010.1

MENFGAVLKDIRISKNFRLKDLSCNEISESTISRFENGITKLSINHFYILLNRLGISFSE  
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>C2 WP\_050252507.1

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LQMIRIKS

LLVFFDSEIRATDEELTFLYDYFFTIDIWGNYELELFSTISTLFPLPLYFKYSREMLQK  
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>AAA26968.1

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LETFASEMINRTQFYNNLPENRRRIKMLLNVSACIENHLQVAMKFLNYIDNTKIP  
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LTVVADLQIE

## SFile 2

>SPD\_0150

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>SPD\_0191

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GAAATCTCATGCTCCAGTTTCGAGGTTTCGTCCAACCAACGAATTATCGATGTGCA  
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>SPD\_0215

GGTTTGGTTCATGACATCGAAGGTAATCAAGATATCAATGATGTCTTCTCAGATA  
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GCTACAAATGTTATACTGAAATAGTCTGACTTATAATTGTTGTCTCTGTGTCTAGA  
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GAAGTTGAAGGCAAAGTAG

>SPD\_0315

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>SPD\_0550

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GGATGGGTATGTGAAAAACATACTTGTGGGAGGTAAAAATCTCTAATTACCGCC  
AAAACCACAAAGGAGGATTTAAAAATGGCTAAAAAAGTCGAAAAACTTGTA  
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>SPD\_0144

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CCCAAGCTCGTGTACTGCCCAAAAAAGTTAGACATTCTATTTAGAACGAGGATTG  
AGTTCTGTATTGTACAAGGCTCGGTCTTTTAGAGTCAGCTTAAGGCTGGCTTTTT  
CAATCACCAAAGTGTGAGAATGTTTTGACAAATGAACACAAATAATGATATAAT  
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>SPD\_1041

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>SPD\_1127

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>SPD\_1370

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GGCGTTTTTTCGAATTCTAGTGTTTTTACTTGCGTAAAAAAATTTTTTCTAGTATA  
ATAGTTTATTGTGAGCGAACCTCACTTACCCCTTGCAAAGTCTTGGGGTCATTAG  
ACCAAAGGAGGAACATATCAATGGCTAAATACGAAATTCTTTATAT

>SPD\_1513

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AATTTGTATGATATACCATCGTTTAGAATAAGTCTATATTTAAAATAGAAGTTAT  
AAGGAATCTTCTTGAATAAAAAATGAAAGGAAACAGAAAAAATGAAAATTAAG  
AAATTATTGAAAATGGTTATTCCTGTTT

>SPD\_2032

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ATCGTCGTGAGGATGGCGAAATTGGTTTGTTAGAGGTTAAAGAATCTTAATTTCA  
ATATGTGTAAAGGTAGGTTTACTGAATTGTAACTACCTTTTTCTTATAATTGATA  
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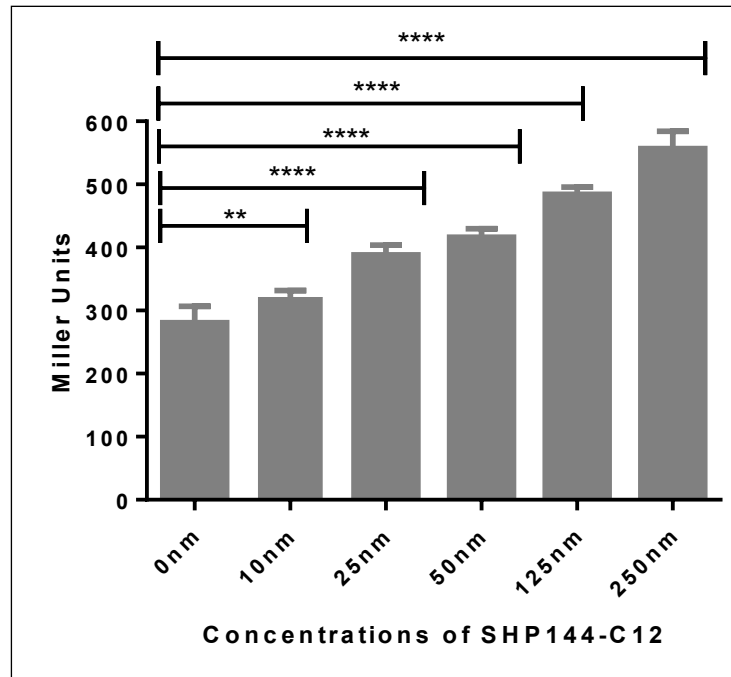
>SPD\_2068

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TAGATTTCAATTAAGAAAATCACAATTTCAAAGAGTTATCCACAGTTTGTGTAA  
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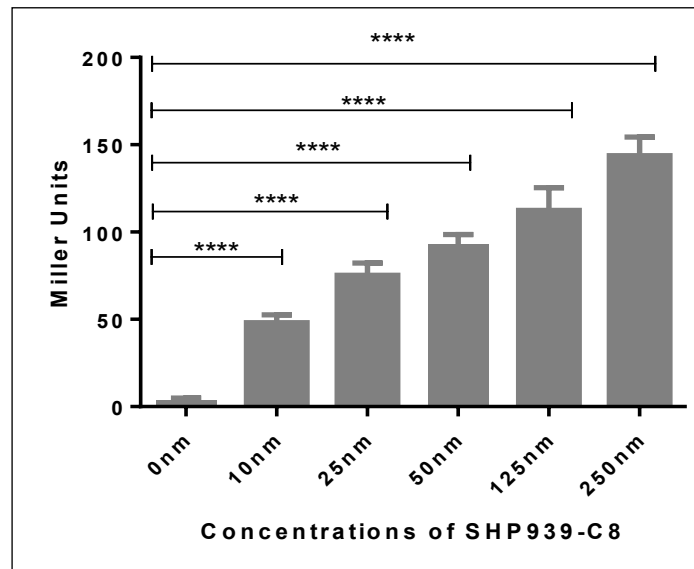
## SFigure 1

		H2	H3	
SGO0496	MLIVKSS	GKILKII	RESKNMSLKEVAAGDISVAQLSRYERGISSLTVDSFYSCLRNMSVS	60
SPD1952	---MEQI	GKVFRQL	RESRNISLRQATGGQFSPSMLSRFETGQSELLVEKFLFALENISAS	57
SPD0999	---MEHL	GKVFRFRT	SGNYSLKEAAGESCSTSQLSRFELGESDLAVSRFFEILDNIHVT	57
SPD0144	MIEKMEL	GEFYKEL	R LARKLKQTDVACEGLTASQLSKFELGQSMLSADKLILAIQGINVT	60
SPD0939	--MKS	KLGVTLRKIR	KGKQISLCSVADEHLSKSQISRFERGESEISCIRLINILDKLHIT	58
SPD1518	--MRWDY	GQIFKEIR	KSKGLTQQDVCGQVIHRTLTNIEHGKVI PSFENMVFLLEQIDMS	58
		*	: : * . . . . : : . * *	
SGO0496	LAEF	QYVYHN---	YREADDVVLSQKLSEAQRENNIVKLESILAGSEAMAQEFPEKKNYKL	117
SPD1952	VEEIL	FLARG---	FQYDTDSELRKEITDVLEPKNIAPLEDLYRREYQKHAHSYNKQKHIL	114
SPD0999	IENF	MDKARN---	FHNHEHVSMMQIIPLYYSNDIAGFQKLQREQLKSKSSTTPLYFEL	114
SPD0144	FDEF	GHKLNN---	YQESPHMRIGRKVVNRFAHQDIAALEQLLEEVDQEQMAQTYRR---	114
SPD0939	LDEF	LILHDEY-	TKTESFANLVQYIRKQYSSQSINNACLLSDTSDYTLNS-----	F 110
SPD1518	LAEF	KYICNEYHPSKRDRDIIVESQNPSTFQDTRKMVELTEKCQKYLKTHHDVPIQNIYRH		118
	. : :	:	... :	
SGO0496	NTIVIRATLTSCNPDYQVSKGDIEFLTDYLFVSVEE	WG-----		154
SPD1952	NAIIKSYMKSIDERVDLTAEEGKVLHDYLFSTEI	WGIYE----		154
SPD0999	NWILLQGLICQRDASYDMKQDDLDKVADYLFKTEE	WTMYE----		154
SPD0144	NAIVIKDAIHSLNKSYPLAEEDSEFLTTYLYAIES	WTWFE----		154
SPD0939	EKTMVKSILHTMDSNIIPSEELLHLTDYLFKIEK	WGYYEIILL		154
SPD1518	TKIVTELRTKGFKNNHVLK-DLYEEIWDYLEPMDT	WY-----		154
	.	:	.	** :

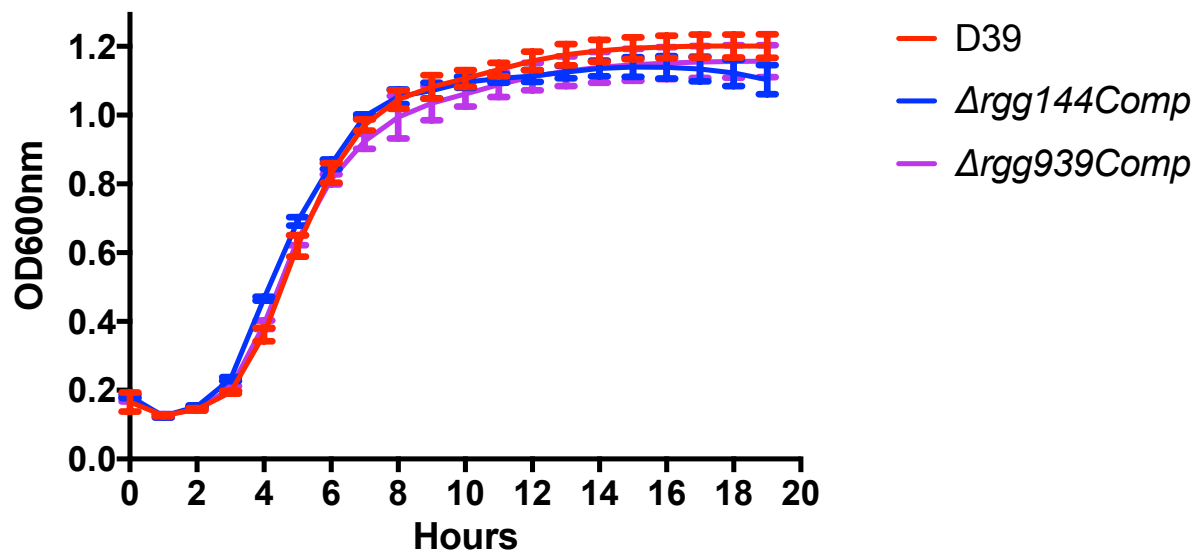
**SFigure 1: Alignment of deduced amino acid sequences of *Streptococcus gordonii* SGO0496 and putative pneumococcal Rgg proteins (SPD1952, SPD0999, SPD0144, SPD0939, and SPD1518).** The primary amino acid sequence of the putative pneumococcal Rggs have been aligned to the *S. gordonii* Rgg. Multiple alignments were performed using ClustalW. The putative helix-turn-helix motif is indicated in bold typeface, including helix 2 (H2) and helix 3 (H3) which are predicted to make contact with target DNA. The conserved residues known to be invariant in most Rgg family members are shown in red. Accession numbers: SGO0496 (AAA26968.1), SPD1952 (ABJ54225.1), SPD0999 (ABJ55078.1), SPD0144 (ABJ54913.1), SPD0939 (ABJ54684.1), and SPD1518 (ABJ55270.1)



**SFigure 2. Expression levels (in Miller units) of *Pshp144-lacZ*-wt in response to different concentrations of synthetic SHP144-C12.** The activity is expressed in nmol *p*-nitrophenol/min/ml. Values are average of three independent experiments each with three replicates.



**SFigure 3. Expression levels (in Miller units) of *Pshp939-lacZ*-wt in response to different concentrations of synthetic SHP939-C8.** The activity is expressed in nmol *p*-nitrophenol/min/ml. Values are average of three independent experiments each with three replicates.



**SFigure 4: Pneumococcal growth curves performed micro-aerobically in CDM supplemented with 1% (w/v) mannose.** Error bars show the standard error of the mean for three individual measurements, each with three replicates (n=9).





**SFigure 5: Analysis of differentially expressed genes and operons to identify a consensus Rgg binding motif.** The upstream regions of differentially expressed operons on galactose and mannose were analysed using Motif-based sequence analysis tool (MEME) to produce a putative Rgg binding motif. The sequence data used to generate the binding motif are provided as SFile 2

**STable 1. List of strains or plasmids used and constructed in this study.**

Strain or plasmid*	Description	Source
D39	Serotype 2 strain	Laboratory collection
$\Delta rgg144$	D39; SPD0144:Spec <sup>R</sup>	this study
$\Delta rgg939$	D39; SPD0939: Kan <sup>R</sup>	this study
$\Delta rgg144/939$	D39; SPD0144:Spec <sup>R</sup> ; SPD0939:Kan <sup>R</sup>	this study
$\Delta shp144$	D39; SHP0144:Spec <sup>R</sup>	this study
$\Delta shp939$	D39; SHP0939: Kan <sup>R</sup>	this study
$\Delta rgg144$ Comp	D39; $rgg144 + \Delta rgg144$ :Spec <sup>R</sup> ; Kan <sup>R</sup>	this study
$\Delta rgg939$ Comp	D39; $rgg939 + \Delta rgg939$ :Spec <sup>R</sup> ; Kan <sup>R</sup>	this study
XZ1	D39; P <sub><i>rgg144</i></sub> - <i>lacZ</i>	this study
XZ2	D39; P <sub><i>shp144</i></sub> - <i>lacZ</i> ; Tet <sup>R</sup>	this study
XZ3	D39; P <sub><i>rgg939</i></sub> - <i>lacZ</i> ; Tet <sup>R</sup>	this study
XZ4	D39; P <sub><i>shp939</i></sub> - <i>lacZ</i> ; Tet <sup>R</sup>	this study
XZ5	$\Delta rgg144$ :Spec <sup>R</sup> ; P <sub><i>rgg144</i></sub> - <i>lacZ</i> ; Tet <sup>R</sup>	this study
XZ6	$\Delta rgg144$ :Spec <sup>R</sup> ; P <sub><i>shp144</i></sub> - <i>lacZ</i> ; Tet <sup>R</sup>	this study

XZ8	$\Delta rgg144$ :Spec <sup>R</sup> ; P <sub>shp939</sub> -lacZ; Tet <sup>R</sup>	this study
XZ10	$\Delta rgg939$ :Kan <sup>R</sup> ; P <sub>shp144</sub> -lacZ; Tet <sup>R</sup>	this study
XZ11	$\Delta rgg939$ :Kan <sup>R</sup> ; P <sub>rgg939</sub> -lacZ; Tet <sup>R</sup>	this study
XZ12	$\Delta rgg939$ :Kan <sup>R</sup> ; P <sub>shp939</sub> -lacZ; Tet <sup>R</sup>	this study
XZ18	$\Delta rgg144/939$ : Spec <sup>R</sup> ; Kan <sup>R</sup> ; P <sub>shp144</sub> -lacZ; Tet <sup>R</sup>	this study
XZ20	$\Delta rgg144/939$ : Spec <sup>R</sup> ; Kan <sup>R</sup> ; P <sub>shp939</sub> -lacZ; Tet <sup>R</sup>	this study
XZ26	$\Delta shp144$ :Spec <sup>R</sup> ;P <sub>shp144</sub> -lacZ ; Tet <sup>R</sup>	this study
XZ36	$\Delta shp939$ :Kan <sup>R</sup> ;P <sub>shp939</sub> -lacZ ; Tet <sup>R</sup>	this study
<i>E.coli</i> BL21 DE3	<i>F-ompT hsdSB (rb- mb-) gal dcm</i> (DE3)	Novagen
<i>E. coli</i> DH5 $\alpha$	Antibiotic sensitive strain used for plasmid propagation	Laboratory collection
pPP2*	Contains promoterless <i>lacZ</i> gene, Amp <sup>R</sup> Tet <sup>R</sup>	Halfmann <i>et al.</i> , 2007
pCEP*	Genetic complementation; Kan <sup>R</sup>	Guiral <i>et al.</i> , 2006
pDL278*	Amplification of Spec <sup>R</sup> ( <i>aadA</i> )	Yesilkaya <i>et al.</i> , 2000

pLEICS-01\*

6His-Tag, Amp<sup>R</sup>, used for  
Rgg144 protein expression

PROTEX, UK

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**STable 2: Synthetic peptides used in this study.**

<b>Peptide</b>	<b>Sequence</b>
SHP144-C8	VIPFLTNL
SHP144-C10	VIVIPFLTNL
SHP144-C11	WVIVIPFLTNL
SHP144-C12	EWVIVIPFLTNL
SHP144-C13	SEWVIVIPFLTNL
SHP144-C13REV	LNTLFPVIVWES
SHP144-C8REV	LNTLFPVIV
SHP144-C14	ISEWVIVIPFLTNL
SHP144-C15	LISEWVIVIPFLTNL
SHP939-C8	DIIIVGG
SHP939C8REV	GGVIIID
SHP939-C9	MDIIIVGG

**STable 3: Oligonucleotide primers used in this study.**

Primers	SEQUENCE
SPD0144-LF	TGGAGGATATTGTCTTCCCAAAG
SPD0144-RR	CTGGGTACGAGGAAAGATATAGGTTG
SPD0144-LR	TATTCACGAACGAAAATCGATTTCATCATCCTACCACCTCC
SPD0144-RF	AACAATAAACCCCTTGCATAACATAGATTAGTTAAAAATGGAA
SHP0144RF	AACAATAAACCCCTTGCATAACCCAGGGAAATAATCAAATCTATCA
specF	ATCGATTTTCGTTTCGTGAATACATGTTAT
specR	GTTATGCAAGGGTTTATTGTTTTCTA
SPD0939RF	TTACTGGATGAATTGTTTTAGCATAGTTTTCCCATTTTCCCA
SPD0939LR	TGCGCGCCATGGTAGCACCTCTAAATGAAATTATTCTCTATC
SPD0939RR	TCCCAATGTACCAATAACTCGAGG
SPD0939LF	ATCCACTGAAGAGATTGAGGAAGC
shp0939rf	TTACTGGATGAATTGTTTTAGGCTTAGGTGTACACCTAAGTC
shp0939rr	CCACTTAGTTGAAGCAGCATC
KanF	GAGGTGCTACCATGGCGCGCATGC
KanR	CTAAAACAATTCATCCAGTAAAATATAATA
Spd0939NcoI	CGCCATGGATTTCACGAGAATGCCTTAC
Spd0939BamH1	ACGGGATCCCTTCATACTCTGACTTCC TT
SHP0939BamH1	ACGGGATCCGATACTCTAAAGACTTAGGT
Spd0144BamH1	ACGGGATCCCGGAGTGGGTATTGTCA
SHP0144BamH1	ACGGGATCCCAAGTACAGTATAACACG
Spd0144NcoI	CGCCATGGCAATCACCAAAGTGTCAG
Prgg144SphI	GACGCATGCTCTTCATACTCTGACTTCC

Prgg144SphI	GAC <b>GCATGC</b> ACGAGGATTGAGTTCTG
Pshp939SphI	GAC <b>GCATG</b> CAATTTGTTTGCCCTTACG
Pshp144BamHI	ACG <b>GGATCC</b> AGTAGGAGTATCAGTTGG
Prgg144BamHI	ACG <b>GGATC</b> CCCAAACGTAATTCCTTG
Prgg939BamHI	ACG <b>GGATCC</b> GTTTGCCCTTACGAATT
Pshp939BamHI	ACG <b>GGATC</b> CTCTTCATACTCTGACTTCC
Shp0144lr	TATTCACGAACGAAAATCGATGTGGGTATTGTCATTCCATTT
Shp0939lr	TGCGCGCCATGGTAGCACCTCTCTTCATACTCTGACTTCCTTTC
Shp144lf	TGCTGTCTGCGGATAAGTTGA
Shp0939lf	CCACTGGTTGATTGGCAAC
malF	GCTTGAAAAGGAGTATACTT
pCEPR	AGGAGACATTCCTTCCGTATC
Fusion-seqR	AGGCGATTAAGTTGGGTAAC
Fusion-seqF	CTACTTGGAGCCACTATCGA

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\*Restriction enzyme sites are in bold. Bold typeface nucleotides are cloning sites

**STable 4. Microarray analysis of gene expression in  $\Delta$ rgg144 relative to wild type D39 grown micro-anaerobically in CDM supplemented with mannose\*.**

Locus	Name	Fold change	Function
SPD_0001	<i>dnaA</i>	2.1	chromosomal replication initiator protein DnaA
SPD_0008		2.43	septum formation initiator, putative
SPD_0015		3.62	
SPD_0017		3.08	
SPD_0020		6.75	
SPD_0046	<i>blpU</i>	2.91	bacteriocin BlpU
SPD_0047		6.79	hypothetical protein
SPD_0093		2.19	hypothetical protein
SPD_0094		2.18	hypothetical protein
SPD_0150		4.5	ABC transporter, substrate-binding protein
SPD_0151		4.04	lipoprotein
SPD_0179		2.71	lipoprotein, putative
SPD_0180		2.83	hypothetical protein
SPD_0181		2.78	conserved hypothetical protein TIGR00250
SPD_0187	<i>nrdD</i>	4.08	anaerobic ribonucleoside-triphosphate reductase
SPD_0189		3.95	acetyltransferase, GNAT family protein
SPD_0191		2.88	hypothetical protein
SPD_0192	<i>rpsJ</i>	2.72	ribosomal protein S10
SPD_0193	<i>rplC</i>	2.47	ribosomal protein L3
SPD_0194	<i>rplD</i>	2.16	ribosomal protein L4
SPD_0198	<i>rplV</i>	2.7	ribosomal protein L22
SPD_0199	<i>rpsC</i>	2.28	ribosomal protein S3
SPD_0200	<i>rplP</i>	2.08	ribosomal protein L16
SPD_0201	<i>rpmC</i>	3.24	ribosomal protein L29
SPD_0202	<i>rpsQ</i>	2.84	ribosomal protein S17
SPD_0203	<i>rplN</i>	2.48	ribosomal protein L14
SPD_0214	<i>adk</i>	2.04	adenylate kinase
SPD_0215	<i>infA</i>	3.17	translation initiation factor IF-1
SPD_0216	<i>rpsM</i>	2.82	ribosomal protein S13
SPD_0217	<i>rpsK</i>	3.11	ribosomal protein S11
SPD_0218	<i>rpoA</i>	3.2	DNA-directed RNA polymerase, alpha subunit



SPD_0219	<i>rplQ</i>	4.54	ribosomal protein L17
SPD_0308	<i>clpL</i>	5.3	ATP-dependent Clp protease, ATP-binding subunit
SPD_0313		2.44	hypothetical protein
SPD_0315	<i>cps2A</i>	2.98	integral membrane regulatory protein Cps2A
SPD_0316	<i>cps2B</i>	2.96	tyrosine-protein phosphatase CpsB
SPD_0317	<i>cps2C</i>	3.07	chain length determinant protein/polysaccharide export protein, MPA1 family protein
SPD_0318	<i>cps2D</i>	3.23	tyrosine-protein kinase Cps2D cytosolic ATPase domain
SPD_0319	<i>cps2E</i>	2.34	undecaprenylphosphate glucosephosphotransferase Cps2E
SPD_0322	<i>cps2G</i>	2.32	glycosyl transferase, group 1 family protein
SPD_0323	<i>csp2H</i>	3.58	Polysaccharide polymerase
SPD_0325		2.62	hypothetical protein
SPD_0326	<i>cps2K</i>	3.13	UDP-glucose 6-dehydrogenase, putative
SPD_0327	<i>cps2P</i>	4.48	UDP-galactopyranose mutase
SPD_0334	<i>aliA</i>	2.12	oligopeptide ABC transporter, oligopeptide-binding protein AliA
SPD_0337	<i>recU</i>	2.07	recombination protein U
SPD_0373		4.6	hypothetical protein
SPD_0420	<i>pflB</i>	2.39	formate acetyltransferase
SPD_0441		2.04	DNA-directed RNA polymerase, delta subunit, putative
SPD_0473	<i>blpY</i>	2.61	immunity protein BlpY
SPD_0474		2.3	hypothetical protein
SPD_0550	<i>rplK</i>	2.67	ribosomal protein L11
SPD_0551	<i>rplA</i>	3.21	ribosomal protein L1
SPD_0577	<i>zmpB</i>	2.44	zinc metalloprotease ZmpB
SPD_0702	<i>ciaH</i>	2.35	sensor histidine kinase CiaH
SPD_0710	<i>ezrA</i>	3.59	septation ring formation regulator EzrA
SPD_0712		3.19	transposase family protein
SPD_0756		3.23	
SPD_0757	<i>rpsA</i>	2.34	ribosomal protein S1
SPD_0835	<i>frr</i>	2.29	ribosome recycling factor
SPD_0899		2.48	
SPD_0905		2.09	acetyltransferase, GNAT family protein

SPD_0915		3.32	iron-compound ABC transporter, iron compound-binding protein
SPD_0961		2.25	glycosyl transferase, group 1
SPD_0989	<i>rplU</i>	2.62	ribosomal protein L21
SPD_1041	<i>nrdH</i>	2.7	glutaredoxin-like protein NrdH
SPD_1042	<i>nrdE</i>	2.56	ribonucleoside-diphosphate reductase, alpha subunit
SPD_1043	<i>nrdF</i>	3.13	ribonucleoside-diphosphate reductase, beta subunit
SPD_1076	<i>srtA</i>	2.24	sortase
SPD_1106		5.83	
SPD_1125	<i>pck</i>	2.12	choline kinase
SPD_1127	<i>ispD</i>	2.16	2-C-methyl-D-erythritol 4-phosphate cytidyltransferase
SPD_1175		2.49	hypothetical protein
SPD_1200		2.11	glycosyl transferase, group 1 family protein
SPD_1297		2.48	pyridoxine biosynthesis protein
SPD_1339	<i>atpF</i>	2.43	ATP synthase F0, B subunit
SPD_1365		2.03	hypothetical protein
SPD_1367		2.25	Cof family protein/peptidyl-prolyl cis-trans isomerase, cyclophilin type
SPD_1368	<i>rpsR</i>	2.71	ribosomal protein S18
SPD_1369	<i>ssb</i>	2.76	single-strand binding protein
SPD_1370	<i>rpsF</i>	2.65	ribosomal protein S6
SPD_1410		5.12	
SPD_1474	<i>divIVA</i>	2.5	cell division protein DivIVA
SPD_1475	<i>ylmH</i>	2.19	YlmH protein
SPD_1513		2.08	
SPD_1514		2.91	ABC transporter, ATP-binding protein
SPD_1515		3.23	hypothetical protein
SPD_1516		4.51	hypothetical protein
SPD_1517		2.17	hypothetical protein
SPD_1522		2.15	hypothetical protein
SPD_1529		2.12	
SPD_1566		2.24	hypothetical protein
SPD_1567	<i>trx</i>	2.28	thioredoxin
SPD_1581		8.85	

SPD_1588		4.88	hypothetical protein
SPD_1589		4.04	lipoprotein, putative
SPD_1590		3.24	general stress protein 24, putative
SPD_1591		4.23	hypothetical protein
SPD_1671	<i>amiA</i>	3.13	oligopeptide ABC transporter, oligopeptide-binding protein AmiA
SPD_1685		4.06	
SPD_1686		10.79	
SPD_1688		7.85	
SPD_1689		4.12	
SPD_1690		2.6	
SPD_1691		8.45	
SPD_1692		4.18	
SPD_1693		3.53	
SPD_1694		5.49	
SPD_1695		2.5	
SPD_1696		3.48	
SPD_1697		2.88	
SPD_1698		6.26	
SPD_1730		2.11	
SPD_1879		4.76	
SPD_1881		2.14	
SPD_1882		4.07	
SPD_1896	<i>gltX</i>	2.45	glutamyl-tRNA synthetase
SPD_1898		6.73	hypothetical protein
SPD_1899		3.33	glutamine amidotransferase, class 1
SPD_1963	<i>rpmF</i>	2.43	ribosomal protein L32
SPD_1965	<i>pcpA</i>	4.56	choline binding protein PcpA
SPD_2020		2.71	DNA-binding response regulator
SPD_2030	<i>dnaB</i>	2.17	replicative DNA helicase
SPD_2031	<i>rplI</i>	2.01	ribosomal protein L9
SPD_2032		2.45	DHH subfamily 1 protein
SPD_2033	<i>yfiA</i>	2.84	ribosomal subunit interface protein
SPD_2037	<i>cysK</i>	3.85	cysteine synthase A
SPD_2043		3.59	secreted 45 kDa protein precursor

SPD_2066	3.62	
SPD_2068	7.35	serine protease
SPD_2069	5.71	SpoJ protein
	<i>adhA</i>	-2.17
SPD_0685	<i>gor</i>	-2.56
	<i>gpmA</i>	-2.43
SPD_0458	<i>hrcA</i>	-2.63
SPD_1138	<i>htpX</i>	-2.34
SPD_0997	<i>hup</i>	-2.06
SPD_1139	<i>lemA</i>	-2.77
SPD_1647	<i>pepA</i>	-2.33
SPD_0261	<i>pepC</i>	-2.16
SPD_1418	<i>pepQ</i>	-2.22
SPD_0445	<i>pgk</i>	-3.92
SPD_1790	<i>rpmH</i>	-2.01
SPD_0145		-6.75
SPD_0146		-2.67
SPD_0147		-2.4
SPD_0339		-2.4
SPD_0465		-2.16
SPD_0582		-3.56
SPD_1295		-2.35
SPD_1320		-2.25
SPD_1415		-2.07
SPD_1646		-2.1
SPD_1984		-2.4

\*Fold changes  $\geq 2$  or  $\leq -2.0$  ( $\Delta$ *rgg144* compared with D39 wild type). All P-values are  $< 0.001$ .

**STable 5. Microarray analysis of gene expression in *Δrgg939* relative to wild type D39 grown micro-anaerobically in CDM supplemented with mannose\*.**

<b>Locus</b>	<b>Name</b>	<b>Fold change</b>	<b>Function</b>
SPD_0001	<i>dnaA</i>	3.09	chromosomal replication initiator protein DnaA
SPD_0007		2.28	S4 domain protein
SPD_0008		5.95	septum formation initiator, putative
SPD_0009		2.62	hypothetical protein
SPD_0010		2.9	hypothetical protein
SPD_0011	<i>tilS</i>	2.17	tRNA(Ile)-lysidine synthetase
SPD_0046	<i>blpU</i>	2.09	bacteriocin BlpU
SPD_0047		5.46	hypothetical protein
SPD_0093		2.61	hypothetical protein
SPD_0120		2.1	hypothetical protein
SPD_0123		2.07	hypothetical protein
SPD_0126	<i>pspA</i>	2.66	pneumococcal surface protein A
SPD_0150		2.22	ABC transporter, substrate-binding protein
SPD_0151		2.92	lipoprotein
SPD_0179		2.8	lipoprotein, putative
SPD_0180		2.61	hypothetical protein
SPD_0181		2.45	conserved hypothetical protein TIGR00250
SPD_0187	<i>nrdD</i>	4.72	anaerobic ribonucleoside-triphosphate reductase
SPD_0189		4.85	acetyltransferase, GNAT family protein
SPD_0191		2.61	hypothetical protein
SPD_0192	<i>rpsJ</i>	2.53	ribosomal protein S10
SPD_0193	<i>rplC</i>	3.19	ribosomal protein L3
SPD_0194	<i>rplD</i>	2.74	ribosomal protein L4
SPD_0195	<i>rplW</i>	2.19	ribosomal protein L23
SPD_0197	<i>rpsS</i>	2.15	ribosomal protein S19
SPD_0198	<i>rplV</i>	3.06	ribosomal protein L22
SPD_0199	<i>rpsC</i>	2.64	ribosomal protein S3
SPD_0200	<i>rplP</i>	2.06	ribosomal protein L16
SPD_0201	<i>rpmC</i>	3.6	ribosomal protein L29
SPD_0202	<i>rpsQ</i>	3.55	ribosomal protein S17
SPD_0203	<i>rplN</i>	2.74	ribosomal protein L14

SPD_0215	<i>infA</i>	2.78	translation initiation factor IF-1
SPD_0216	<i>rpsM</i>	2.59	ribosomal protein S13
SPD_0217	<i>rpsK</i>	2.96	ribosomal protein S11
SPD_0218	<i>rpoA</i>	2.71	DNA-directed RNA polymerase, alpha subunit
SPD_0219	<i>rplQ</i>	5.03	ribosomal protein L17
SPD_0251	<i>rpsL</i>	2.2	ribosomal protein S12
SPD_0256		2.37	conserved hypothetical protein TIGR00053
SPD_0257		2.97	hypothetical protein
SPD_0308	<i>clpL</i>	2.18	ATP-dependent Clp protease, ATP-binding subunit
SPD_0313		3.35	hypothetical protein
SPD_0315	<i>cps2A</i>	3.15	integral membrane regulatory protein Cps2A
SPD_0316	<i>cps2B</i>	2.68	tyrosine-protein phosphatase CpsB
SPD_0317	<i>cps2C</i>	3.42	chain length determinant protein/polysaccharide export protein, MPA1 family protein
SPD_0318	<i>cps2D</i>	2.9	tyrosine-protein kinase Cps2D cytosolic ATPase domain
SPD_0319	<i>cps2E</i>	2.2	undecaprenylphosphate glucosephosphotransferase Cps2E
SPD_0320	<i>cps2T</i>	2.32	glycosyl transferase, group 1 family protein, putative
SPD_0322	<i>cps2G</i>	2.6	glycosyl transferase, group 1 family protein
SPD_0323	<i>csp2H</i>	4.44	Polysaccharide polymerase
SPD_0325		3.32	hypothetical protein
SPD_0326	<i>cps2K</i>	3.18	UDP-glucose 6-dehydrogenase, putative
SPD_0327	<i>cps2P</i>	4.9	UDP-galactopyranose mutase
SPD_0334	<i>aliA</i>	2.15	oligopeptide ABC transporter, oligopeptide-binding protein AliA
SPD_0337	<i>recU</i>	2.46	recombination protein U
SPD_0342		2.03	hypothetical protein
SPD_0365	<i>tig</i>	2.15	trigger factor
SPD_0373		2.19	hypothetical protein
SPD_0400		2.04	Glycosyl transferase family protein 8, putative
SPD_0422		2.11	hypothetical protein
SPD_0451		2.47	type I restriction-modification system, S subunit, putative
SPD_0460	<i>dnaK</i>	2.14	chaperone protein DnaK

SPD_0493		2.25	hypothetical protein
SPD_0548		2.09	HIT family protein
SPD_0550	<i>rplK</i>	2.59	ribosomal protein L11
SPD_0551	<i>rplA</i>	3.58	ribosomal protein L1
SPD_0558	<i>prtA</i>	2.83	cell wall-associated serine protease PrtA
SPD_0577	<i>zmpB</i>	2.16	zinc metalloprotease ZmpB
SPD_0692		2.36	hypothetical protein
SPD_0710	<i>ezrA</i>	4.12	septation ring formation regulator EzrA
SPD_0712		2.02	transposase family protein
SPD_0726		2.35	purine nucleoside phosphorylase, family protein 2
SPD_0728		2.23	hypothetical protein
SPD_0750		2.48	hypothetical protein
SPD_0757	<i>rpsA</i>	2.35	ribosomal protein S1
SPD_0784		2.87	type I restriction-modification system, R subunit, putative
SPD_0793		2.43	hypothetical protein
SPD_0794		2.12	
SPD_0835	<i>frr</i>	3.24	ribosome recycling factor
SPD_0847	<i>infC</i>	2.25	translation initiation factor IF-3
SPD_0863	<i>smpB</i>	2.22	SsrA-binding protein
SPD_0876		2.02	hypothetical protein
SPD_0878		2.22	hypothetical protein
SPD_0905		3.72	acetyltransferase, GNAT family protein
SPD_0915		9.31	iron-compound ABC transporter, iron compound-binding protein
SPD_0916		2.14	iron-compound ABC transporter, permease protein
SPD_0917		2.27	iron-compound ABC transporter, permease protein
SPD_0919		2.24	hypothetical protein
SPD_0920		3	hypothetical protein
SPD_0932		3.1	hypothetical protein
SPD_0940		2.46	UDP-N-acetyl-D-mannosaminuronic acid dehydrogenase, putative
SPD_0941		5.92	hypothetical protein
SPD_0942		4.56	hypothetical protein

SPD_0945		3.69	AMP-binding enzyme, putative
SPD_0949		2.44	bacterial transferase hexapeptide (three repeats), putative
SPD_0950		2.06	transporter, major facilitator family protein
SPD_0961		2.82	glycosyl transferase, group 1
SPD_0963		2.49	hypothetical protein
SPD_0968		2.7	acetyltransferase, GNAT family protein
SPD_0969		2.4	hypothetical protein
SPD_0972		2.45	IS1381, transposase OrfB
SPD_1041	<i>nrdH</i>	3.75	glutaredoxin-like protein NrdH
SPD_1042	<i>nrdE</i>	2.56	ribonucleoside-diphosphate reductase, alpha subunit
SPD_1043	<i>nrdF</i>	2.31	ribonucleoside-diphosphate reductase, beta subunit
SPD_1055		2.43	
SPD_1064		2.6	hemolysin A, putative
SPD_1077	<i>gyrA</i>	2.09	DNA gyrase, A subunit
SPD_1080		2.52	type II restriction endonuclease, putative
SPD_1083	<i>vicX</i>	2.16	vicX protein
SPD_1084		2.64	sensory box sensor histidine kinase
SPD_1115	<i>leuB</i>	2.01	3-isopropylmalate dehydrogenase
SPD_1125	<i>pck</i>	2.75	choline kinase
SPD_1126		2.07	alcohol dehydrogenase, zinc-containing
SPD_1127	<i>ispD</i>	2.72	2-C-methyl-D-erythritol 4-phosphate cytidyltransferase
SPD_1129	<i>licD1</i>	2.59	phosphotransferase LicD1
SPD_1167		2.31	ABC transporter, ATP-binding protein
SPD_1175		7.3	hypothetical protein
SPD_1177		2.92	drug efflux ABC transporter, ATP-binding/permease protein
SPD_1199		2.18	glycosyl transferase, group 2 family protein
SPD_1200		2.66	glycosyl transferase, group 1 family protein
SPD_1297		2.42	pyridoxine biosynthesis protein
SPD_1338	<i>atpH</i>	3.47	ATP synthase F1, delta subunit
SPD_1339	<i>atpF</i>	3.77	ATP synthase F0, B subunit
SPD_1340	<i>atpB</i>	2.43	ATP synthase F0, A subunit



SPD_1346		3.25	Uncharacterized BCR, putative
SPD_1350		2.14	hypothetical protein
SPD_1367		2.42	Cof family protein/peptidyl-prolyl cis-trans isomerase, cyclophilin type
SPD_1368	<i>rpsR</i>	2.25	ribosomal protein S18
SPD_1369	<i>ssb</i>	2.17	single-strand binding protein
SPD_1370	<i>rpsF</i>	2.78	ribosomal protein S6
SPD_1404	<i>tpiA</i>	2.23	triosephosphate isomerase
SPD_1422		2.21	hypothetical protein
SPD_1463		2.63	ABC transporter, substrate binding lipoprotein
SPD_1474	<i>divIVA</i>	2.8	cell division protein DivIVA
SPD_1475	<i>ylmH</i>	2.06	YlmH protein
SPD_1495		2.3	sugar ABC transporter, sugar-binding protein
SPD_1504	<i>nanA</i>	3.29	sialidase A precursor
SPD_1513		3.43	
SPD_1514		3.53	ABC transporter, ATP-binding protein
SPD_1515		3.95	hypothetical protein
SPD_1516		4.97	hypothetical protein
SPD_1517		2.19	hypothetical protein
SPD_1522		3.37	hypothetical protein
SPD_1529		2.14	
SPD_1558		2.08	conserved hypothetical protein TIGR00253
SPD_1581		2.13	
SPD_1588		4.54	hypothetical protein
SPD_1589		4.53	lipoprotein, putative
SPD_1590		2.34	general stress protein 24, putative
SPD_1591		3.4	hypothetical protein
SPD_1595		4.34	hypothetical protein
SPD_1642	<i>proWX</i>	2.02	choline transporter (glycine betaine transport system permease protein)
SPD_1643	<i>proV</i>	2.22	choline transporter
SPD_1644		2.27	hypothetical protein
SPD_1671	<i>amiA</i>	3.62	oligopeptide ABC transporter, oligopeptide-binding protein AmiA
SPD_1688		2.41	
SPD_1707		2.06	hypothetical protein

SPD_1730		3.11	
SPD_1739	<i>recA</i>	2.84	recA protein
SPD_1873		2.27	hypothetical protein
SPD_1896	<i>gltX</i>	4.03	glutamyl-tRNA synthetase
SPD_1898		6.78	hypothetical protein
SPD_1899		2.19	glutamine amidotransferase, class 1
SPD_1906		2.1	IS1381, transposase OrfB
SPD_1963	<i>rpmF</i>	2.43	ribosomal protein L32
SPD_1965	<i>pcpA</i>	4.49	choline binding protein PcpA
SPD_1967		2.11	IS1381, transposase OrfB
SPD_1981		2.6	hypothetical protein
SPD_2017	<i>cbpA</i>	2.42	choline binding protein A
SPD_2019		2.06	sensor histidine kinase
SPD_2020		4.35	DNA-binding response regulator
SPD_2031	<i>rplI</i>	2.02	ribosomal protein L9
SPD_2032		2.19	DHH subfamily 1 protein
SPD_2043		3.98	secreted 45 kDa protein precursor
SPD_2059		5.41	
SPD_2060		2.89	transcriptional regulator, TetR family protein
SPD_2068		4.11	serine protease
SPD_2069		3.52	SpoJ protein
SPD_0039		-2.24	hypothetical protein
SPD_0116		-2.85	hypothetical protein
SPD_0145		-2.45	hypothetical protein
SPD_0146		-2.64	CAAX amino terminal protease family protein
SPD_0147		-2.18	CAAX amino terminal protease family protein
SPD_0213	<i>secY</i>	-2.01	preprotein translocase, SecY subunit
SPD_0276		-2.05	hypothetical protein
SPD_0425		-2.01	hypothetical protein
SPD_0442	<i>pyrG</i>	-2.12	CTP synthase
SPD_0458	<i>hrcA</i>	-2.98	heat-inducible transcription repressor HrcA
SPD_0459	<i>grpE</i>	-2.09	heat shock protein GrpE
SPD_0463		-2.06	Hit-like protein involved in cell-cycle regulation, putative
SPD_0485		-2.03	hypothetical protein

SPD_0511	<i>metF</i>	-2.15	5,10-methylenetetrahydrofolate reductase
SPD_0582		-2.38	hypothetical protein
SPD_0588		-2.09	transcriptional regulator, putative
SPD_0646		-2.15	hypothetical protein
SPD_0770		-2.62	
SPD_0772		-2.09	1-phosphofructokinase, putative
SPD_1004	<i>gapN</i>	-2.12	glyceraldehyde-3-phosphate dehydrogenase, NADP-dependent
SPD_1138	<i>htpX</i>	-2.26	heat shock protein HtpX
SPD_1139	<i>lemA</i>	-2.19	LemA protein
SPD_1255		-2.11	ABC transporter, ATP-binding protein
SPD_1354		-2.04	hypothetical protein
SPD_1415		-2.01	oxidoreductase, pyridine nucleotide-disulfide, class I
SPD_1506		-2.07	acetyl xylan esterase, putative
SPD_1663	<i>treC</i>	-2.06	alpha,alpha-phosphotrehalase
SPD_1682		-3.83	
SPD_1683		-2.13	
SPD_1687		-2.38	
SPD_1735		-2.31	hypothetical protein
SPD_1797	<i>ccpA</i>	-2.19	catabolite control protein A
SPD_1834		-2.41	alcohol dehydrogenase, iron-containing
SPD_1843		-2.05	hexulose-6-phosphate isomerase, putative
SPD_1917		-2.14	hypothetical protein
SPD_1932	<i>malP</i>	-3	maltodextrin phosphorylase
SPD_1933	<i>malQ</i>	-2.43	4-alpha-glucanotransferase
SPD_1956	<i>ilvD</i>	-2.08	dihydroxy-acid dehydratase
SPD_1984		-2.56	hypersensitive-induced reaction protein 4
SPD_2006		-2.31	hypothetical protein
SPD_2009		-2.02	hypothetical protein

\*Fold changes  $\geq 2$  or  $\leq -2.0$  ( $\Delta$ rgg939 compared with D39 wild type). All P-values are  $<0.001$ .

**STable 6. Microarray analysis of gene expression in *Δrgg144* relative to wild type D39 grown micro-anaerobically in CDM supplemented with galactose\*.**

Locus	Name	Fold change	Function
SPD_0015		2.87	
SPD_0020		2.17	
SPD_0116		2.05	hypothetical protein
SPD_0121		1.92	hypothetical protein
SPD_0451		2.27	type I restriction-modification system, S subunit, putative
SPD_0756		2.35	
SPD_0899		2.9	
SPD_1581		2.27	
SPD_1682		4.73	
SPD_1687		2	
SPD_1688		1.99	
SPD_1691		2.03	
SPD_1692		2.22	
SPD_1694		2.12	
SPD_1695		2.31	
SPD_1696		3.13	
SPD_1879		4.56	
SPD_1881		2.33	
SPD_0144		-2.47	hypothetical protein
SPD_0145		-6.39	CAAX amino terminal protease family protein
SPD_0146		-5.49	CAAX amino terminal protease family protein
SPD_0147		-3.92	transporter, major facilitator family protein
SPD_0148		-3.65	hypothetical protein
SPD_0149		-1.83	ABC transporter, ATP-binding protein
SPD_0900	<i>asd</i>	-2.32	transcriptional regulator
SPD_1514		-2.04	hypothetical protein
SPD_1515		-1.99	hypothetical protein
SPD_1516		-2.41	

\*Fold changes  $\geq 2$  or  $\leq -2.0$  (*Δrgg144* compared with D39 wild type). All P-values are  $< 0.001$ .

**STable 7. Microarray analysis of gene expression in  $\Delta$ rgg939 relative to wild type D39 grown micro-anaerobically in CDM supplemented with galactose\*.**

Locus	Name	Fold change	Function
SPD_0089		2.17	ABC transporter, permease protein
SPD_0116		2.4	hypothetical protein
SPD_0121		2.64	hypothetical protein
SPD_0146		2.6	CAAX amino terminal protease family protein
SPD_0147		2.36	CAAX amino terminal protease family protein
SPD_0451		2.26	type I restriction-modification system, S subunit, putative
SPD_0473	<i>blpY</i>	2.13	immunity protein BlpY
SPD_0913		2	hypothetical protein
SPD_0940		1.84	UDP-N-acetyl-D-mannosaminuronic acid dehydrogenase, putative
SPD_0941		4.4	hypothetical protein
SPD_0942		4.72	hypothetical protein
SPD_0945		3.4	AMP-binding enzyme, putative
SPD_0946		2.65	hypothetical protein
SPD_0949		4.14	bacterial transferase hexapeptide (three repeats), putative
SPD_0950		2.61	transporter, major facilitator family protein
SPD_1588		2.34	hypothetical protein
SPD_2069		2.56	SpoJ protein
SPD_1513		-2.59	
SPD_1514		-2.92	ABC transporter, ATP-binding protein
SPD_1515		-2.2	hypothetical protein
SPD_1516		-2.78	hypothetical protein

\*Fold changes  $\geq 2$  or  $\leq -2.0$  ( $\Delta$ rgg939 compared with D39 wild type). All P-values are  $<0.001$ .

**STable 8. Fold difference in expression of selected genes in  $\Delta rgg144$  and  $\Delta rgg939$  relative to wild type D39 measured by quantitative reverse transcriptase PCR (qRT-PCR) and microarray analysis.** The pneumococcal strains were grown micro-anaerobically in CDM supplemented with mannose. The mean and standard deviation (in parenthesis) for three individual measurements have been provided.

Locus	Name	Fold difference in expression of genes regulated by Rgg144		Fold difference in expression of genes regulated by Rgg939		Function
		qRT-PCR	Microarray	qRT-PCR	Microarray	
SPD_0008		3.56 (0.17)	2.43	4.45 (0.26)	5.96	septum formation initiator, putative
SPD_0198	<i>rplV</i>	2.7 (0.11)	3.95	3.06 (0.15)	2.46	ribosomal protein L22
SPD_0219	<i>rplQ</i>	4.54 (0.24)	3.92	5.03 (0.20)	6.81	ribosomal protein L17
SPD_0318	<i>cps2D</i>	3.23 (0.14)	2.89	2.9 (0.10)	3.10	tyrosine-protein kinase <i>Cps2D</i> cytosolic ATPase domain
SPD_0550	<i>rplK</i>	2.67 (0.08)	5.59	2.59 (0.09)	1.21	ribosomal protein L11
SPD_1041	<i>nrdH</i>	2.7 (0.10)	1.91	3.75 (0.16)	2.89	glutaredoxin-like protein NrdH
SPD_1474	<i>divIVA</i>	2.5 (0.07)	3.62	2.8 (0.08)	2.34	cell division protein DivIVA