

A. Number of differentially expressed genes

	10T vs 9T ⁽¹⁾		11T vs 9T ⁽²⁾		11T vs 10T ⁽³⁾		Growth phase
	Number of genes	% ⁽⁴⁾	Number of genes	%	Number of genes	%	
Upregulated	18	1%	59	3.2%	8	0.4%	ME
Downregulated	13	0.7%	28	1.5%	7	0.4%	
Upregulated	102	5.6%	12	0.7%	8	0.4%	ES
Downregulated	154	8.4%	98	5.4%	5	0.3%	

(1) MGAS2761-10T compared to MGAS2761-9T

(2) MGAS2761-11T compared to MGAS2761-9T

(3) MGAS2761-11T compared to MGAS2761-10T

(4) Percentage of genes compared to the entire genome

B. Upregulated virulence genes (10T vs 9T)

	Growth	Spy	gene	Product	FC
1	ME	Spy0137	<i>nga</i>	NAD glycohydrolase	1.5
2	ME	Spy0138	<i>ifs</i>	immunity factor for SPN	1.6
3	ME	Spy0139	<i>slo</i>	streptolysin O	1.6
4	ME	Spy0540	<i>sagA</i>	streptolysin S precursor	1.6
5	ME	Spy1336	<i>Spy1336</i>	R28 protein	64.7
6	ME	Spy1337	<i>Spy1337</i>	transcriptional regulator, AraC family	4.3
7	ME	Spy1675	<i>sclA</i>	collagen-like surface protein A	2.4
8	ME	Spy1699	-	cell surface protein	1.6
9	ME	Spy1700	<i>scpA</i>	CSA peptidase precursor	1.7
10	ME	Spy1701	<i>enn</i>	enn protein	1.5
11	ME	Spy1702	<i>emm</i>	emm28 protein	1.7
1	ST	Spy0138	<i>ifs</i>	immunity factor for SPN	1.6
2	ST	Spy0139	<i>slo</i>	streptolysin O	1.5
3	ST	Spy1336	<i>Spy1336</i>	R28 protein	45.3
4	ST	Spy1337	<i>Spy1337</i>	transcriptional regulator, AraC family	7.6

C. Upregulated virulence genes (11T vs 9T)

	Growth	Spy	gene	Product	FC
1	ME	Spy0137	<i>nga</i>	NAD glycohydrolase	1.8
2	ME	Spy0138	<i>ifs</i>	immunity factor for SPN	2.0
3	ME	Spy0139	<i>slo</i>	streptolysin O	1.9
4	ME	Spy0329	<i>spyCEP</i>	lactocepin	2.9
5	ME	Spy0540	<i>sagA</i>	streptolysin S precursor	1.7
6	ME	Spy0541	<i>sagB</i>	streptolysin S biosynthesis protein	1.6
7	ME	Spy0542	<i>sagC</i>	streptolysin S biosynthesis protein	1.6
8	ME	Spy0543	<i>sagD</i>	streptolysin S biosynthesis protein	1.7
9	ME	Spy0544	<i>sagE</i>	streptolysin S putative self-immunity protein	1.8
10	ME	Spy0545	<i>sagF</i>	streptolysin S biosynthesis protein	1.7
11	ME	Spy0546	<i>sagG</i>	streptolysin S export ATP-binding protein	1.8
12	ME	Spy0547	<i>sagH</i>	streptolysin S export transmembrane protein	1.7
13	ME	Spy0548	<i>sagI</i>	streptolysin S export transmembrane protein	1.8
14	ME	Spy1336	<i>Spy1336</i>	R28 protein	115.0
15	ME	Spy1337	<i>Spy1337</i>	transcriptional regulator, AraC family	6.0
16	ME	Spy1675	<i>sclA</i>	collagen-like surface protein A	3.7
17	ME	Spy1699	-	cell surface protein	2.1
18	ME	Spy1700	<i>scpA</i>	CSA peptidase precursor	2.2
19	ME	Spy1701	<i>enn</i>	enn protein	1.9
20	ME	Spy1702	<i>emm</i>	emm28 protein	1.9
1	ST	Spy0137	<i>nga</i>	NAD glycohydrolase	1.9
2	ST	Spy0138	<i>ifs</i>	immunity factor for SPN	1.8
3	ST	Spy0139	<i>slo</i>	streptolysin O	1.7
4	ST	Spy1336	<i>Spy1336</i>	R28 protein	72.6
5	ST	Spy1337	<i>Spy1337</i>	transcriptional regulator, AraC family	9.1
6	ST	Spy1702	<i>emm</i>	emm28 protein	2.3
7	ST	Spy1716	<i>saf</i>	serum opacity factor	1.5

D. Upregulated virulence genes (10T vs 11T)

	Growth	Spy	gene	Product	FC
1	ME	Spy0329	<i>spyCEP</i>	lactocepin	1.5
2	ME	Spy1336	<i>Spy1336</i>	R28 protein	1.8
1	ST	Spy1336	<i>Spy1336</i>	R28 protein	1.6
2	ST	Spy1675	<i>sclA</i>	collagen-like surface protein A	1.7
3	ST	Spy1702	<i>emm</i>	emm28 protein	1.7
4	ST	Spy1621	<i>salA</i>	lantibiotic salivaricin A	1.8

Supplementary Figure 2. RNA-seq results comparing isogenic 9T, 10T, and 11T

strains. (A) Total number of differentially expressed genes using a fold cutoff ≥ 1.5 .

Upregulated virulence genes comparing 9T vs 10T (B), 9T vs 11T (C), and 10T vs 11T

(D).