

**S1 Table. Independently validated sporulation genes identified by Tn-seq.**

Gene	<i>p</i> -value	Tn. fold change $\Delta\text{ssdC}/\text{WT}^{\text{a}}$	Possible product	Spo. in $\Delta\text{ssdC}$ (% $\pm$ STDEV) <sup>b</sup>	Spo. fold change / $\Delta\text{ssdC}^{\text{c}}$
<b><i>ssdC</i></b>	-	-	RseB-like protein required for spore shape	3.21 $\pm$ 1.71	-
<b><i>cotE</i></b>	3.20 x 10 <sup>-4</sup>	333.3	Outer spore coat morphogenetic protein	0.16 $\pm$ 1.09	20.1
<b><i>cotZ</i></b>	4.20 x 10 <sup>-4</sup>	71.4	Spore crust protein, necessary for the assembly of the spore crust	2.61 $\pm$ 1.31	1.23
<b><i>ctpB</i></b>	< 10 <sup>-7</sup>	37.0	Processing serine protease, cleaves SpoIVFA.	0.90 $\pm$ 0.29	3.58
<b><i>dltB</i></b>	< 10 <sup>-7</sup>	2.3	D-alanine transfer from DltC to undecaprenol-phosphate	1.27 $\pm$ 0.63	2.52
<b><i>dltD</i></b>	1.66 x 10 <sup>-4</sup>	4.0	D-alanine transfer from undecaprenol-phosphate to the poly(glycerophosphate) chain	1.80 $\pm$ 0.72	1.79
<b><i>mcsA</i></b>	1.20 x 10 <sup>-5</sup>	6.9	Modulator of CtsR dependent repression	1.18 $\pm$ 0.50	2.73
<b><i>rpsT</i></b>	4.13 x 10 <sup>-3</sup>	333.3	Ribosomal protein	2.99 $\pm$ 1.80	1.07
<b><i>safA</i></b>	< 10 <sup>-7</sup>	25.6	Spore coat morphogenetic protein and major organizer of the inner spore coat	0.07 $\pm$ 0.02	48.3
<b><i>skfA</i></b>	< 10 <sup>-7</sup>	2.6	Spore killing factor	2.41 $\pm$ 0.01	1.33
<b><i>spoVID</i></b>	1.50 x 10 <sup>-4</sup>	250	Spore coat morphogenetic protein, promotes encasement of the spore	0.09 $\pm$ 0.03	36.2
<b><i>yacL</i></b>	1.40 x 10 <sup>-4</sup>	3.2	Unknown	0.46 $\pm$ 0.20	7.04
<b><i>yceE</i></b>	1.90 x 10 <sup>-2</sup>	4.2	Unknown	1.76 $\pm$ 1.08	1.82
<b><i>yjgD</i></b>	2.32 x 10 <sup>-2</sup>	6.8	Unknown	2.55 $\pm$ 0.55	1.26
<b><i>yImD</i></b>	2.00 x 10 <sup>-5</sup>	5.6	Peptidoglycan-editing factor	3.43 $\pm$ 0.90	0.94
<b><i>ymzB</i></b>	7.55 x 10 <sup>-3</sup>	2.6	Unknown	2.17 $\pm$ 1.41	1.48
<b><i>yochH</i></b>	3.00 x 10 <sup>-6</sup>	3.3	Peptidoglycan hydrolase	1.82 $\pm$ 0.23	1.77
<b><i>yodJ</i></b>	1.31 x 10 <sup>-2</sup>	2.3	LD-carboxypeptidase	0.42 $\pm$ 0.37	7.59
<b><i>ypbE</i></b>	2.33 x 10 <sup>-3</sup>	2.3	Peptidoglycan-binding protein	3.22 $\pm$ 0.81	1.00
<b><i>yqfT</i></b>	4.02 x 10 <sup>-2</sup>	125	Unknown	0.94 $\pm$ 0.50	3.43
<b><i>yqhG</i></b>	6.00 x 10 <sup>-5</sup>	333	Unknown	1.19 $\pm$ 0.76	2.69
<b><i>yqhH</i></b>	1.00 x 10 <sup>-5</sup>	6.0	Similar to sucrose non-fermenting 2 helicase	1.21 $\pm$ 1.07	2.65
<b><i>yqhQ</i></b>	4.40 x 10 <sup>-4</sup>	3.4	Unknown	2.14 $\pm$ 0.23	1.50
<b><i>yrrI</i></b>	1.21 x 10 <sup>-3</sup>	4.0	Similar to coordinator of zonal elongation	0.54 $\pm$ 0.35	5.92
<b><i>yrrL</i></b>	2.00 x 10 <sup>-6</sup>	1.9	Potential terminase for peptidoglycan polymerization	2.77 $\pm$ 0.55	1.16
<b><i>yteV</i></b>	8.81 x 10 <sup>-4</sup>	250	Unknown	2.33 $\pm$ 0.76	1.38
<b><i>yxiS</i></b>	2.79 x 10 <sup>-2</sup>	2.1	Unknown	2.51 $\pm$ 1.75	1.28

**Legend:**

<sup>a</sup> Fold-difference in the number of transposon insertions: e.g. the  $\Delta\text{ssdC}$  mutant had 333.3-fold less transposon insertions than the WT.

<sup>b</sup> Sporulation efficiency, relative to WT, of  $\Delta\text{ssdC}$  double mutants with the listed genes: e.g. the  $\Delta\text{ssdC} \Delta\text{cotE}$  double mutant had a sporulation efficiency of 0.16 %. The data is average of two biological replicates.

<sup>c</sup> Fold-change in sporulation efficiency of the  $\Delta\text{ssdC}$  double mutants with the listed genes, relative to the  $\Delta\text{ssdC}$  single mutant: the  $\Delta\text{ssdC} \Delta\text{cotE}$  mutant produced 20.1-fold less spores than the  $\Delta\text{ssdC}$  single mutant.