

**Supplemental Information for:**

**Identification of an autorepressing two-component signaling system that modulates virulence in *Streptococcus suis* serotype 2**

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**Supplemental Table S1. Differentially expressed genes in the  $\Delta TCS09HKRR$  strain versus WT strain**

Gene/locus name	Function or description	log2 Fold Change	p-value	q-value
<i>ZY05719_00870</i>	molecular chaperone GroEL	-1.0405	1.13E-24	1.01E-22
<i>ZY05719_01235</i>	thioredoxin	-1.1314	5.26E-06	9.48E-05
<i>ZY05719_02230</i>	PTS fructose transporter subunit IIC	-1.0463	0.012503	0.049462
<i>ZY05719_02235</i>	PTS fructose transporter subunit IID	-1.1408	0.00306	0.017383
<i>cps2A</i>	LytR family transcriptional regulator	-2.8175	6.72E-75	1.20E-72
<i>cps2B</i>	capsular biosynthesis protein CpsC	-3.0338	1.26E-93	2.74E-91
<i>cps2C</i>	tyrosine protein kinase	-2.9877	1.53E-62	2.50E-60
<i>cps2D</i>	tyrosine protein phosphatase	-3.2172	5.84E-50	8.81E-48
<i>cps2E</i>	galactosyl transferase	-2.9235	2.42E-29	2.64E-27
<i>cps2F</i>	glycosyl transferase	-2.974	3.67E-35	4.50E-33
<i>cps2G</i>	glycosyl transferase family 1	-2.9174	3.26E-27	3.37E-25
<i>cps2H</i>	glycosyl transferase	-2.7401	4.78E-19	3.61E-17
<i>cps2I</i>	polymerase	-2.718	9.88E-24	8.08E-22
<i>cps2J</i>	glycosyl transferase	-2.7782	4.61E-30	5.31E-28
<i>cps2K</i>	glycosyl transferase	-2.8808	7.10E-81	1.39E-78
<i>ZY05719_02805</i>	membrane protein	-3.1185	3.37E-157	2.20E-154
<i>ZY05719_02830</i>	lipooligosaccharide sialyltransferase	-3.0466	7.66E-105	3.01E-102
<i>ZY05719_02835</i>	capsid assembly protein	-2.9467	8.93E-117	4.38E-114
<i>neuB</i>	N-acetyl neuramic acid synthetase NeuB	-2.2941	3.97E-102	1.30E-99
<i>neuC</i>	UDP-N-acetylglucosamine 2-epimerase	-2.204	2.17E-95	5.32E-93
<i>neuD</i>	NeuD protein	-2.2597	1.99E-96	5.58E-94

<i>neuA</i>	acylneuraminate cytidyltransferase	-2.2983	1.54E-49	2.16E-47
ZY05719_02860	transposase	-2.29	2.01E-37	2.63E-35
ZY05719_02865	transposase	-2.5799	2.67E-16	1.80E-14
ZY05719_03810	phosphoribosyl transferase	-1.2901	2.51E-24	2.14E-22
ZY05719_03815	aspartate carbamoyltransferase catalytic subunit	-1.0469	6.92E-21	5.43E-19
ZY05719_03965	PTS fructose transporter subunit IIC	-1.4535	0.004108	0.021713
ZY05719_04270	antitoxin	-1.0531	0.002388	0.014407
ZY05719_04895	dihydroorotate dehydrogenase	-1.0679	1.65E-16	1.16E-14
ZY05719_04900	dihydroorotate dehydrogenase	-1.1242	4.34E-18	3.15E-16
ZY05719_05820	preprotein translocase subunit YajC	-1.4315	1.14E-05	0.000186
ZY05719_05830	PTS system N-acetylgalactosamine-specific transporter subunit IIC	-1.0804	0.011796	0.047313
ZY05719_07065	6-phospho-beta-glucosidase	-1.3878	4.81E-07	1.12E-05
ZY05719_07070	PTS beta-glucoside transporter subunit IIABC	-1.4273	9.57E-11	4.36E-09
ZY05719_07075	transcription antiterminator BglG	-1.4656	3.15E-09	1.17E-07
ZY05719_09180	uracil permease	-1.1167	7.08E-26	6.95E-24
ZY05719_09295	glycerol transporter	-1.2933	0.009319	0.040076
ZY05719_09640	carbonate dehydratase	-1.0936	2.20E-25	2.06E-23
ZY05719_09785	amylopullulanase	-1.1716	0.000111	0.001214
ZY05719_00315	multidrug ABC transporter ATPase	1.3881	0.00111	0.0078293
ZY05719_00650	tRNA-Leu	1.3	0.000434	0.0037674
ZY05719_00655	integrase	1.2458	0.005216	0.02598
ZY05719_00665	Cro/C1 family transcriptional regulator	1.5613	0.000964	0.007002
ZY05719_00770	competence protein CglA	2.0128	1.10E-05	0.00018148

ZY05719_00775	competence protein CglB	2.198	8.70E-06	0.00014714
ZY05719_00800	hypothetical protein	2.8635	0.004559	0.023529
ZY05719_01015	6-phospho-beta-glucosidase	1.3375	3.75E-08	1.10E-06
ZY05719_01650	adhesion protein	1.2416	1.37E-09	5.37E-08
ZY05719_01670	tRNA-Leu	1.0607	0.001904	0.012005
ZY05719_02900	transcriptional regulator	1.9006	3.82E-05	0.00052013
ZY05719_03485	pyridine nucleotide-disulfide oxidoreductase	1.3402	6.10E-06	0.00010689
ZY05719_03495	glycerol kinase	1.1965	0.003365	0.018745
ZY05719_03550	phosphomethylpyrimidine kinase	1.0338	0.003347	0.018714
ZY05719_04460	conjugal transfer protein	2.4518	3.34E-06	6.43E-05
ZY05719_04475	ATP/GTP-binding protein	1.9111	2.19E-08	6.91E-07
ZY05719_04495	Cro/C1 family transcriptional regulator	1.5743	0.002286	0.013923
ZY05719_04500	cell division protein FtsK	1.8085	0.00294	0.016855
ZY05719_04670	hypothetical protein	1.3726	9.42E-09	3.24E-07
ZY05719_04685	glucan-binding protein	1.3364	3.69E-07	8.94E-06
ZY05719_04700	amidase	1.0905	0.000357	0.003204
ZY05719_04705	ATPase AAA	1.7164	1.54E-06	3.27E-05
ZY05719_04715	conjugal transfer protein TrbL	1.874	0.001808	0.011474
ZY05719_04725	conjugal transfer protein TraG	1.9138	9.09E-05	0.0010485
ZY05719_04765	modification methylase	1.5773	0.009722	0.041267
ZY05719_04775	replication initiator protein	2.3006	9.79E-08	2.67E-06
ZY05719_05075	hypothetical protein	1.2374	0.000718	0.005478
ZY05719_05585	2-dehydro-3-deoxygluconokinase	1.3898	0.000643	0.0050605
ZY05719_05590	glucuronide permease	1.0105	0.010717	0.044273
ZY05719_06890	type I restriction-modification protein subunit S	1.7054	7.90E-06	0.00013479
ZY05719_07190	DNA primase	1.2634	1.38E-05	0.00021591

<i>ZY05719_07215</i>	hypothetical protein	1.7578	0.006491	0.031046
<i>ZY05719_07220</i>	hypothetical protein	1.6353	0.009048	0.039255
<i>ZY05719_09065</i>	sugar ABC transporter substrate-binding protein	1.042	0.001031	0.0073
<i>ZY05719_09080</i>	N-acetyl-beta-D-glucosaminidase	1.442	1.76E-07	4.60E-06
<i>ZY05719_09085</i>	ROK family transcriptional regulator	1.45	0.000913	0.0067063
<i>ZY05719_09090</i>	alpha-mannosidase	1.5202	7.35E-10	3.07E-08
<i>ZY05719_09095</i>	glycosyl hydrolase	1.4175	1.36E-05	0.00021536
<i>ZY05719_09105</i>	endo-beta-N-acetylglucosaminidase	1.0435	8.73E-11	4.07E-09
<i>ZY05719_09850</i>	diacetylchitobiose-6-phosphate hydrolase	1.1952	4.46E-06	8.26E-05

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**Supplemental Table S2. Bacterial strains and plasmids used in this study**

Strains or plasmids	Description <sup>a</sup>	Source or reference
Bacterial strains		
ZY05719	The SS2 strain was isolated from a diseased pig	Our laboratory
$\Delta TCS09HKRR$	Deletion mutant of <i>TCS09HKRR</i> with ZY05719 background	This study
$C\Delta TCS09HKRR$	$\Delta TCS09HKRR$ with the vector pSET2- <i>TCS09HKRR</i> , Spc <sup>r</sup>	This study
$\Delta TCS09HK$	Deletion mutant of <i>TCS09HK</i> with ZY05719 background	This study
$\Delta TCS09RR$	Deletion mutant of <i>TCS09RR</i> with ZY05719 background	This study
$\Delta TCS09HK-HAMP$	Deletion mutant of TCS09HK HAMP domain with ZY05719 background	This study
<i>E. coli</i> DH5 $\alpha$	Cloning host for maintaining the recombinant plasmids	Invitrogen
<i>E. coli</i> BL21 (DE3)	Host for expressing the recombinant proteins	Invitrogen
Plasmids		
pSET4S	Temperature-sensitive suicide vector for <i>S. suis</i> mutagenesis, Spc <sup>r</sup>	(1)
pSET4S- <i>TCS09HKRR</i>	Derived from pSET4S used to knock out <i>TCS09HKRR</i> in ZY05719, Spc <sup>r</sup>	This study
pSET4S- <i>TCS09HK</i>	Derived from pSET4S used to knock out <i>TCS09HK</i> in ZY05719, Spc <sup>r</sup>	This study
pSET4S- <i>TCS09RR</i>	Derived from pSET4S used to knock out <i>TCS09RR</i> in ZY05719, Spc <sup>r</sup>	This study
pSET4S- <i>TCS09HK-HAMP</i>	Derived from pSET4S used to knock out <i>TCS09HK-HAMP</i> in ZY05719, Spc <sup>r</sup>	This study
pSET2	<i>E. coli</i> - <i>S. suis</i> shuttle vector for <i>S. suis</i> complementation, Spc <sup>r</sup>	(2)
pSET2- <i>TCS09HKRR</i>	pSET2 containing the promoter followed by the full-length <i>TCS09HKRR</i> ORF, Spc <sup>r</sup>	This study
pCold II	His-tag expressing vector, Amp <sup>r</sup>	Invitrogen
pCold II- <i>TCS09RR</i>	pCold II inserted in-frame with the <i>TCS09RR</i> gene for expressing TCS09RR, Amp <sup>r</sup>	This study

<sup>a</sup>Spc<sup>r</sup>, spectinomycin resistant; Amp<sup>r</sup>, Ampicillin resistant.

**Supplemental Table S3. Primers used in this study**

Primers	Primers sequence (5'-3')
For deletion	
Del-TCS09hkrr-Upstream-F	GAGTCAGA <u>AATTC</u> CTTACCATCCATCCGTTTAG
Del-TCS09hkrr-Upstream-R	GCACAGGAAAAG GGTTGGTTA
Del-TCS09hkrr-Downstream-F	CTTTTCCTGTGC CGGCACCTTTCTTTTTTCAA
Del-TCS09hkrr-Downstream-R	CTGATC <u>GTCGAC</u> AAGGCCTTATCCAGTCCTAC
Del-TCS09rr-Upstream-F	GAGTCAGA <u>AATTC</u> CTTACCATCCATCCGTTTAG
Del-TCS09rr-Upstream-R	GCACAGGAAAAG GGTTGGTTA
Del-TCS09rr-Downstream-F	CTTTTCCTGTGC TTGGTCTTCTGCGACTAAGAT
Del-TCS09rr-Downstream-R	CTGATC <u>GTCGAC</u> TTTTGGGGCAATCTGCAATTT
Del-TCS09hk-Upstream-F	GAGTCAGA <u>AATTC</u> GCGCCTAGCTTGGACAGGATA
Del-TCS09hk-Upstream-R	CGAATTCGGTTT GAGGAGGTG
Del-TCS09hk-Downstream-F	AAACGGAATTCG CGGCACCTTTCTTTTTTCAA
Del-TCS09hk-Downstream-R	CTGATC <u>GTCGAC</u> AAGGCCTTATCCAGTCCTAC
Del-TCS09hk-HAMP-Upstream-F	GAGTCAGA <u>AATTC</u> GACTAGCTCCAAAGGTTTGGG
Del-TCS09hk-HAMP-Upstream-R	AAGCGGGAAGCC CTCAGCAA
Del-TCS09hk-HAMP-Downstream-F	GGCTTCCCGCTT GACCATGCCCACGAAGAACAT
Del-TCS09hk-HAMP-Downstream-R	CTGATC <u>GTCGAC</u> TATTGAGCAGGTTTGGGCTAG
For identification	
TCS09rr-identification-F	CTAACCAACCCTTTTCCTGTG
TCS09rr-identification-R	GCAATGCTGCGGGACGCCCTC
TCS09hk-identification-F	GGCATCTGCTAGCCTGACTGT
TCS09hk-identification-R	TTTTGGGGCAATCTGCAATTT
TCS09hk-HAMP-identification-F	GTGGATAACAAGTGCAAAAGC

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TCS09hk-HAMP-identification-R	TATTGAGCAGGTTTGGGCTAG
For complementation	
TCS09hkrr complementation-F	<u>CGGGATCC</u> ATGCCCAATCTCATTCGTTTA
TCS09hkrr complementation-R	AA <u>CTGCAG</u> TGACATTTGTCACATGAGTCC
For TCS09RR expression	
TCS09rr pCold-F	<u>CGGAATTC</u> TTATACTAACCAACCCTTTTCCTG
TCS09rr pCold-R	CG <u>GGATCC</u> ATGAGAATCTTAGTCGCAGAAGAC
For checking <i>TCS09hkrr</i> operon	
rr-hk-F	TCTTTTTCCAAAAGAGCAATG
rr-hk-R	GATTTTACAGCAGATGTTGGA
hk-30-F	CCAAAACTCATGACGGCGAT
hk-30-R	GCAAATCCAGTCTGAACAGGC
30-35-F	CAAAGCCCGACATGGAAAAAC
30-35-R	TACGGCGGAGCGGATTTTGGT
For EMSA	
TCS09hkrr(D1/D2/D3) EMSA-F	GACTTCGTTTCCTAGGCTACT
TCS09hkrr(D1/D2/D3) EMSA-R	TTTTCTACCTCCTACTTCTTG
cps EMSA-F	ATTATGACCAAGAAGTTGGTG
cps EMSA-R	TATTCAAAGCCATCCACCTTA
neu EMSA-F	CTTCCACCAATCTATTGAAGA
neu EMSA-R	ATTACAACCAATTTCTGCAAT
16s EMSA-F	CGAGCGCAGGCGGTTTGATAA

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16s EMSA-R	CTCATCGTTTACGGCGTGGAC
D1-Upstream-F	AGATGGATGAATATATGGAAGAAA
D1-Upstream-R	ATTATACTCAAT GAAAATCAAAAG
D1-Downstream-F	ATTGAGTATAAT CAAAGGCTTCTA GTGGGGTCT
D1-Downstream-R	TGTCGATTTCCACATCTGTCA
D2-Upstream-F	AGATGGATGAATATATGGAAGAAA
D2-Upstream-R	ATTATACTCAAT GAAAATCAAAAG
D2-Downstream-F	ATTGAGTATAAT ATGAGTCCATGACAAAGGCTT
D2-Downstream-R	TGTCGATTTCCACATCTGTCA
D3-Upstream-F	AGATGGATGAATATATGGAAGAAA
D3-Upstream-R	GGACTCATGTGA CAAATGTCATAT
D3-Downstream-F	TCACATGAGTCC CAAAGGCTTCTA GTGGGGTCT
D3-Downstream-R	TGTCGATTTCCACATCTGTCA
D1/D2/D3-overlap extension-F	TGGAAGAAATTGATGGTTATT
D1/D2/D3-overlap extension-R	ATCCTTGTAATAGCTTGGAA
For quantitative real-time reverse transcription-PCR	
TCS09rr (08020) qPCR-F	CCTCTGTCAGCTCTTGTGTT
TCS09rr (08020) qPCR-R	CACCACATCTACTGGCTCTTT
TCS09hk (08025) qPCR-F	CTATGCCCAGTATAGTCCCAATC
TCS09hk (08025) qPCR-R	GAATAGAGCTTGTGGTCGGTATAA
08030 qPCR-F	CTGGCACTACTACCTATCCAAAC
08030 qPCR-R	CCTGAGCTGACAAATCTACTCC
08035 qPCR-F	GGCTCAGGGTGTGACTATTT
08035 qPCR-R	AGCAAATGGCGTGGTATCT
07580 qPCR-F	CATCCCGTGTGCCCATAT

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07580 qPCR-R	GCCAGGAGAACCGTCATT
06495 qPCR-F	GTGACCAAGCCCTTCTCTAATC
06495 qPCR-R	CAAATCGCCAATCACCAACTC
04260 qPCR-F	ACATGGGTGGCGATGATTT
04260 qPCR-R	CGCTCGATTTGCTGAATTGATTA
09630 qPCR-F	GCAACAAGTTAGTCGTCCCT
09630 qPCR-R	ATCTTGGTTCCATCCGTTTCA
10050 qPCR-F	GAGCAGGTCTATCTTGTGGAAC
10050 qPCR-R	CAAGCGTAGGTTGGAAGTATAGG
00770 qPCR-F	GCCATCATCAGCCATTTCAAG
00770 qPCR-R	AAATCTCTCCGCTCCCATAATC
06890 qPCR-F	TGGGAAGATTCCGAGAAATTGG
06890 qPCR-R	CGCACACCTCTTTGGATGATAG
09105 qPCR-F	GGGAGATGACGGTGAAAGATAC
09105 qPCR-R	CAATGCCTTCTCCACCTTCT
02760 (cps2C) qPCR-F	GCCTATGCTCGTTCAGGTTAT
02760 (cps2C) qPCR-R	CTGTTGTCCCTGCTAGGTAATC
02775 (cps2F) qPCR-F	CTTGTCGGATTGGTCCTTTCA
02775 (cps2F) qPCR-R	CAATACTGTTCGGACGGGATAAC
02840 (neuB) qPCR-F	TAGCACAGATATGCCCGTTTAT
02840 (neuB) qPCR-R	ACAGCCATACCAGTTGAAAGA
07070 qPCR-F	GGCCTACTCAGCTTCACAAA
07070 qPCR-R	GAAGTGGATCACCTGAAGATACC
01650 qPCR-F	ACTCAACACACAGCCTTCTC
01650 qPCR-R	CAACTGTCTAGGACTTGGTTCTT
04460 qPCR-F	GGATTCAGATATGGACGGTAGAG

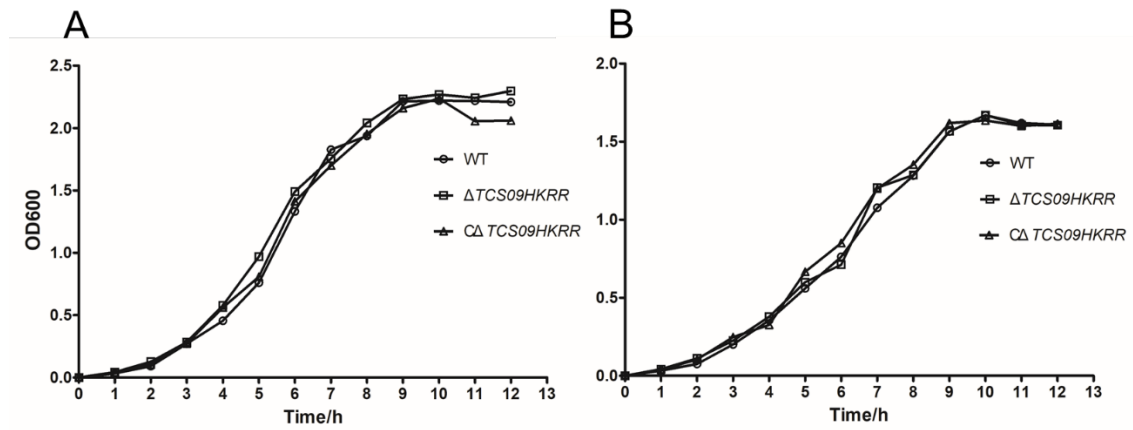
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04460 qPCR-R	CAGAGTGGACGGTCTTTGTATT
01235 qPCR-F	GGCGTAGTCCTTGTTGACTT
01235 qPCR-R	ATCCATCTTCGTCCACTTCATC
09295 qPCR-F	CCCAATCATCGTGGGACTTATC
09295 qPCR-R	GCATGATACGAGGGCCTAAAT
<i>parC</i> qPCR-R	TGGAGATGCACGGAAACAA
<i>parC</i> qPCR-R	CTCGATGTCAGCCAAGAGATAG

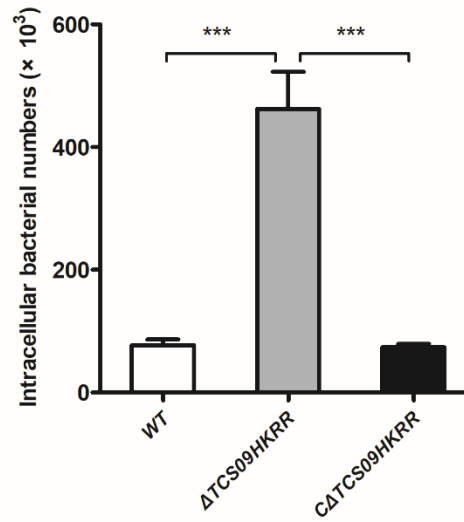
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Underlined are restriction cutting sites.

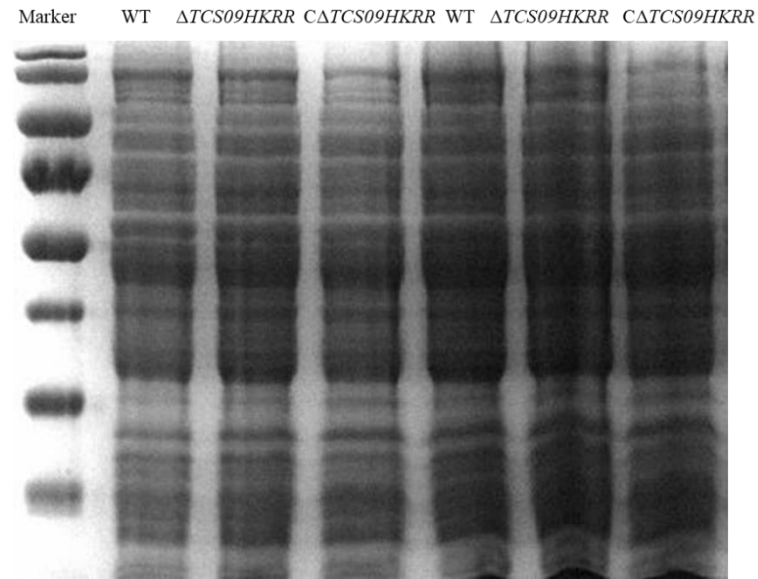


**Supplemental Figure S1 Growth kinetics of WT,  $\Delta TCS09HKRR$  and  $C\Delta TCS09HKRR$  strains in THB under 37°C (A) and 25°C (B). OD<sub>600</sub>**

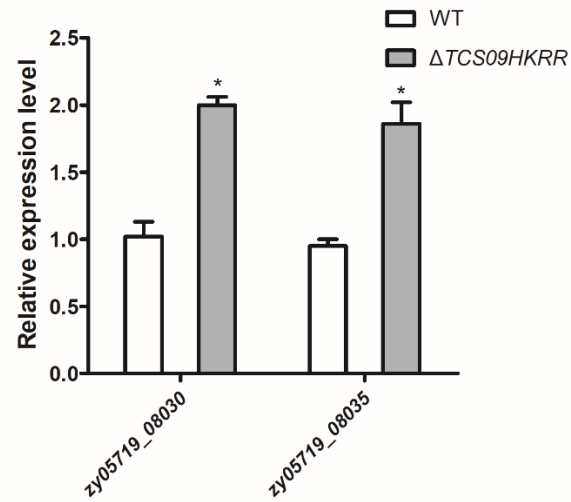
values are the means from three independent experiments.



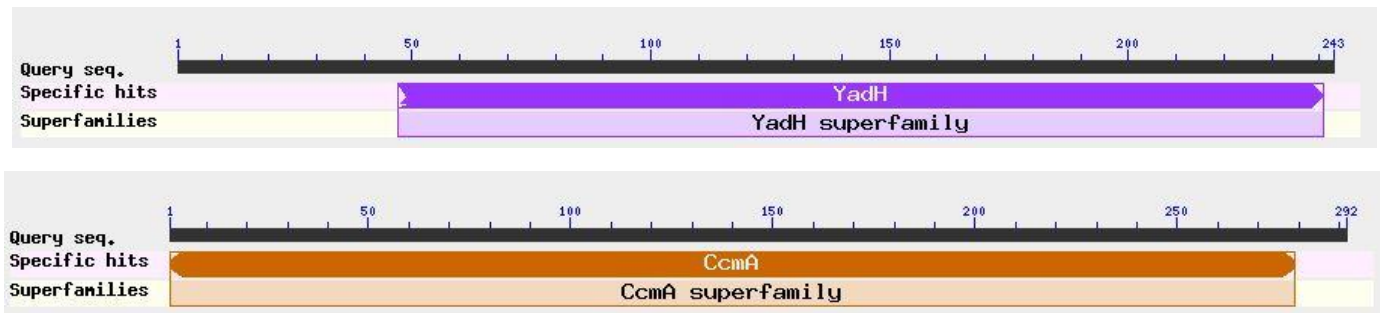
**Supplemental Figure S2 Effect of the *TCS09HKRR* deletion on the ability of SS2 to resist 3D4/21 cells phagocytosis.** At 1.5 h postinfection, cells were washed and incubated in DMEM containing antibiotics (penicillin and gentamicin) for another 1.5 h. Then the cells were washed again and lysed to enumerate the CFU.



**Supplemental Figure S3** Surface proteins of WT,  $\Delta TCS09HKRR$ , and  $C\Delta TCS09HKRR$  were compared in SDS-PAGE analysis. The protein quantity in right 3 lanes were double than that in left 3 lanes.

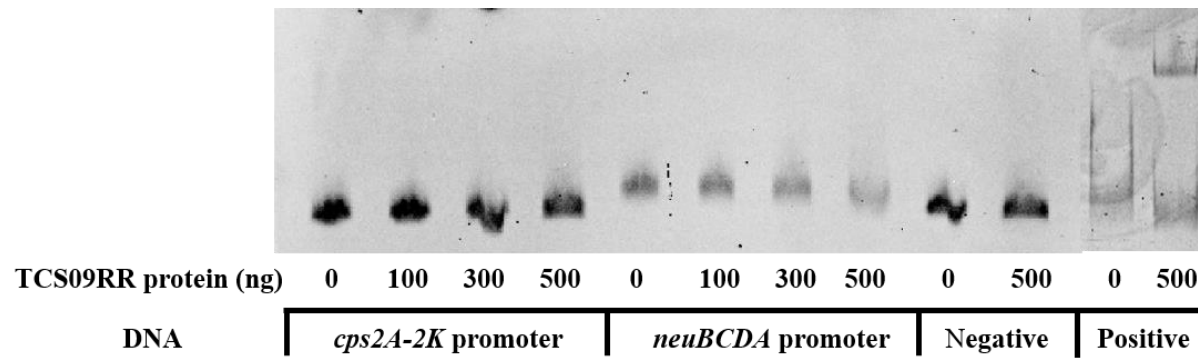


**Supplemental Figure S4** Transcription levels of *zy05719\_08030*, and *zy05719\_08035* genes in WT and  $\Delta TCS09HKRR$  strains were measured by qRT-PCR. qRT-PCR results are represented as means  $\pm$  SD from three independent experiments. Asterisks indicate significant differences (\*,  $P < 0.05$ ).

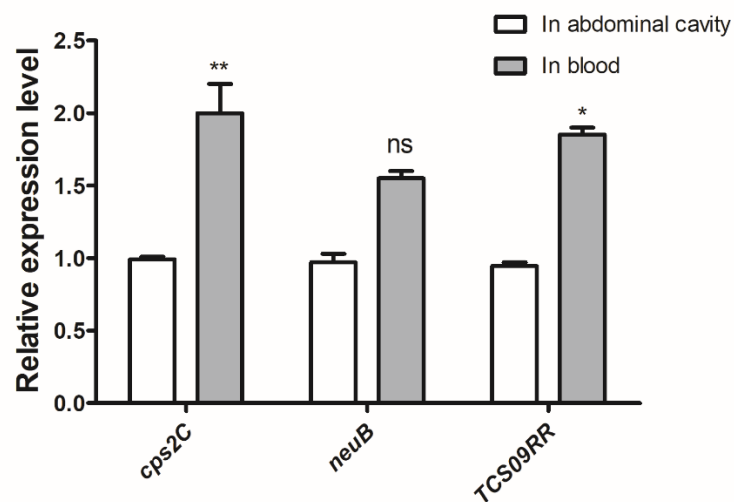


**Supplemental Figure S5 Homology analyses of the ZY05719\_08030 and ZY05719\_08035 proteins by the protein BLAST algorithm. The ZY05719\_08030 show to have putative conserved domain YadH, and ZY05719\_08035 show to have putative conserved domain CcmA.**





**Supplemental Figure S6** An EMSA showed that TCS09RR indirectly regulated the CPS synthesis clusters. The recombinant TCS09RR protein with increasing amounts was added to the reaction mixture. The *TCS09HKRR* promoter region were used as the positive control, and fragments amplified from 16S rRNA served as the negative control.



**Supplemental Figure S7** Transcription levels of *cps2C*, *neuB* and *TCS09RR* in host before and after SS2 entering blood circulation were measured by qRT-PCR. qRT-PCR results are represented as means  $\pm$  SD from three independent experiments. Asterisks indicate significant differences (ns,  $P > 0.05$ ; \*,  $P < 0.05$ ; \*\*,  $P < 0.01$ ).

## References

1. Takamatsu D, Osaki M, Sekizaki T. 2001. Thermosensitive suicide vectors for gene replacement in *Streptococcus suis*. *Plasmid* 46:140-8.
2. Takamatsu D, Osaki M, Sekizaki T. 2001. Construction and characterization of *Streptococcus suis*-*Escherichia coli* shuttle cloning vectors. *Plasmid* 45:101-13.