

SpoVG Regulates Cell Wall Metabolism and Oxacillin Resistance in  
Methicillin-Resistant *Staphylococcus aureus* Strain N315

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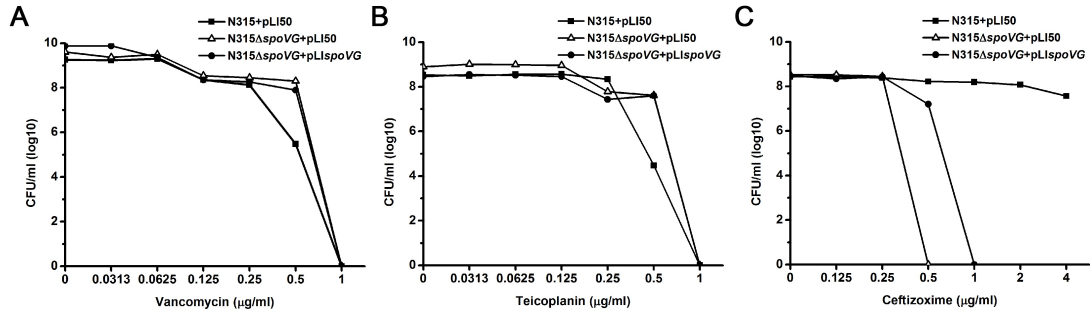
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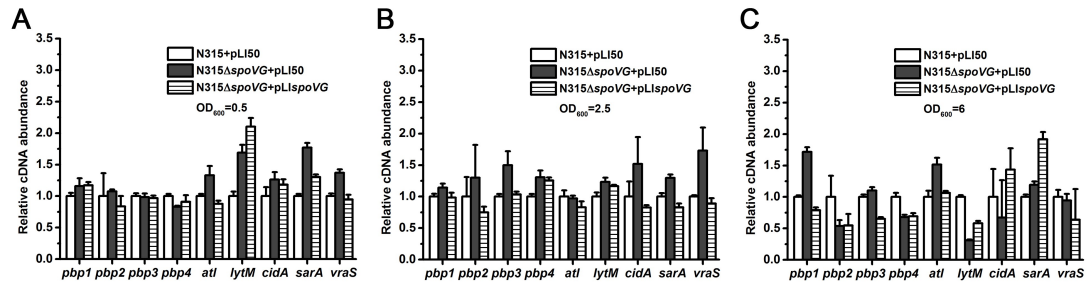
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Running title: SpoVG Regulates Oxacillin Resistance

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**Figure S1. Antibiotic susceptibility assay.** The antibiotic resistance levels were evaluated by population analysis profiles as recommended by the Clinical and Laboratory Standards Institute. The colonies were counted after incubation at 37°C for 24 hours. (A) and (B) There is no dramatic difference in vancomycin and teicoplanin resistance between the *spoVG* mutant (N315Δ*spoVG*+pLI50) and wide-type (N315+pLI50) strains. (C) Deletion of *spoVG* reduced ceftizoxime resistance in strain N315 and the complementary (N315Δ*spoVG*+pLI*spoVG*) strain has a partial effect.



**Figure S2. Transcriptional analysis of cell wall-related genes.** Comparison of the relative transcription levels of several cell wall biosynthesis- and hydrolysis-related genes in the wild-type (N315+pLI50), the *spoVG* mutant (N315Δ*spoVG*+pLI50), and the *spoVG* complementary (N315Δ*spoVG*+pLI*spoVG*) strains. (A), (B), and (C) The relative transcription levels of *pbp1*, *pbp2*, *pbp3*, *pbp4*, *atl*, *lytM*, *cidA*, *sarA*, and *vraS* genes in early exponential (OD<sub>600</sub>=0.5), mid-logarithmic (OD<sub>600</sub>=2.5) and early stationary (OD<sub>600</sub>=6) phases were not significantly affected in the *spoVG* mutant (fold change < 2) except for *lytM* of early stationary phase.

Table S1. Primers used in this study

Primer name	Oligonucleotide <sup>a</sup> (5'-3')	Application
<i>spoVG</i> -mutant-u p-F	GCGggtaccAATTGCCAGTATTTACATGG	pBTsΔ <i>spoVG</i> , pLI <i>spoVG</i>
<i>spoVG</i> -mutant-u p-R	<u>GCAAATGTATTACATCGCTACCCCCCTAT</u> AGTATATATCTC	pBTsΔ <i>spoVG</i>
<i>spoVG</i> -mutant-d own-F	TAGCGATGTAATACATTTGC	pBTsΔ <i>spoVG</i>
<i>spoVG</i> -mutant-d own-R	GCGgtcgacTATTCACCTTGCGCATTATC	pBTsΔ <i>spoVG</i>
P <i>spoVG</i> -R	ACTAAACTCCTTTTATGAAAAC	pLI <i>spoVG</i>
<i>spoVG</i> -complem ent-F	<u>TTCATAAAAGGAGTTTTAGTATGAAAGT</u> GACAGATGTAAGACT	pLI <i>spoVG</i>
<i>spoVG</i> -complem ent-R	GCGggtaccTGCCGACAAAGCTTTATGCT	pLI <i>spoVG</i>
SpoVG-exp-F	CATGccatgggAATGAAAGTGACAGATGTA AGAC	pETSpovG
SpoVG-exp-R	CCGctcgagAGCTTCTTCTGAATCTTCTGAT GTAGC	pETSpovG
RTQ- <i>hu</i> -F	AAAAAGAAGCTGGTTCAGCAGTAG	qRT-PCR
RTQ- <i>hu</i> -R	TTTACGTGCAGCACGTTAC	qRT-PCR

Table S1-continued

Primer name	Oligonucleotide <sup>a</sup> (5'-3')	Application
RTQ- <i>lytN</i> -F	TGACACCATTAGTAGAACCA	qRT-PCR
RTQ- <i>lytN</i> -R	CATTGCCATCCATAACTACC	qRT-PCR
RTQ- <i>lrgA</i> -F	TTATGCCAATTCCTATGC	qRT-PCR
RTQ- <i>lrgA</i> -R	CGAAGAGTAAGCCAATG	qRT-PCR
RTQ- <i>arlS</i> -F	CGAATGATAACACAGTGAGAG	qRT-PCR
RTQ- <i>arlS</i> -R	CTTGCGTTGTAATTGGTTCT	qRT-PCR
RTQ- <i>lytS</i> -F	GGCACGAGAGTTACTATTAGA	qRT-PCR
RTQ- <i>lytS</i> -R	GCACGCACTTGACTTAAC	qRT-PCR
RTQ- <i>sleI</i> -F	CACCAGTATTCAGTCACCAA	qRT-PCR
RTQ- <i>sleI</i> -R	CAGTTATTAGCATTCCACCAAT	qRT-PCR
RTQ- <i>mecA</i> -F	CGACTTCACATCTATTAGGTTAT	qRT-PCR
RTQ- <i>mecA</i> -R	ACACGATAGCCATCTTCA	qRT-PCR
RTQ- <i>femA</i> -F	ACACAACAAGCGAGATAAC	qRT-PCR
RTQ- <i>femA</i> -R	CAGCAGAGATAGGTAATTCATT	qRT-PCR
RTQ- <i>blaZ</i> -F	TTTCCATTTGCGATAAGTTT	qRT-PCR
RTQ- <i>blaZ</i> -R	TCCAGTTAGATATGAGATAGAAT	qRT-PCR
RTQ- <i>lytM</i> -F	CATTCGTAGATGCTCAAGGA	qRT-PCR
RTQ- <i>lytM</i> -R	CTCGCTGTGTAGTCATTGT	qRT-PCR
RTQ- <i>cidA</i> -F	GCAGTCATTATCATAGGAA	qRT-PCR
RTQ- <i>cidA</i> -R	CATTCATAAGCGTCTACA	qRT-PCR

Table S1-continued

Primer name	Oligonucleotide <sup>a</sup> (5'-3')	Application
RTQ- <i>atl</i> -F	AGCACCAACGGATTAC	qRT-PCR
RTQ- <i>atl</i> -R	CATACTCAGCACTGTCT	qRT-PCR
RTQ- <i>sarA</i> -F	GTGCATTAACAAGAATTAATACAGT	qRT-PCR
RTQ- <i>sarA</i> -R	TTGACATACATCAGCGAAAAC	qRT-PCR
RTQ- <i>pbp2</i> -F	ATGGTGTATCTGGAGTAA	qRT-PCR
RTQ- <i>pbp2</i> -R	CATAGTGTATTGAGGTGTAA	qRT-PCR
RTQ- <i>pbp4</i> -F	TATGAACAATAAGTGCTAATCC	qRT-PCR
RTQ- <i>pbp4</i> -R	GACCTCCAACGTAGAAAG	qRT-PCR
RTQ- <i>vraS</i> -F	TGGTCTAATGGTGGTTCT	qRT-PCR
RTQ- <i>vraS</i> -R	CTTCACGATTCTGTTAGTCA	qRT-PCR
<i>plytN</i> -F	AGTTAAAAGATGATATAAAT	EMSA
<i>plytN</i> -R-biotin	AGCGTACTTTACTTTGTTGT	EMSA
<i>plytN</i> -R	AGCGTACTTTACTTTGTTGT	EMSA
<i>pfemA</i> -F	TAATATGAGCAAACCCAAACG	EMSA
<i>pfemA</i> -R-biotin	AGGCACCAAACCTCTTTAGC	EMSA
<i>pfemA</i> -R	AGGCACCAAACCTCTTTAGC	EMSA
<i>plytSR</i> -F	GATTAGAAACTGTTGGCAAAG	EMSA
<i>plytSR</i> -R-biotin	TTGTTAGCGATAGCACGATA	EMSA
<i>plytSR</i> -R	TTGTTAGCGATAGCACGATA	EMSA

Table S1-continued

Primer name	Oligonucleotide <sup>a</sup> (5'-3')	Application
RTQ- <i>pbp1</i> -F	AGTCAGCGACCAACATT	qRT-PCR
RTQ- <i>pbp1</i> -R	AAGCACCTTCTTGAATAGC	qRT-PCR
RTQ- <i>pbp3</i> -F	TTCAGCCGTATCCAACATT	qRT-PCR
RTQ- <i>pbp3</i> -R	GTGCCAAGAGGTCGTATT	qRT-PCR

<sup>a</sup> Lowercase letters indicate restriction sites. Underlined letters indicate overlap sequences used for SLiCE.