Quorum sensing-independent cellulase-sensitive pellicle formation is critical for colonization of *Burkholderia glumae* in rice plants

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Table of Contents

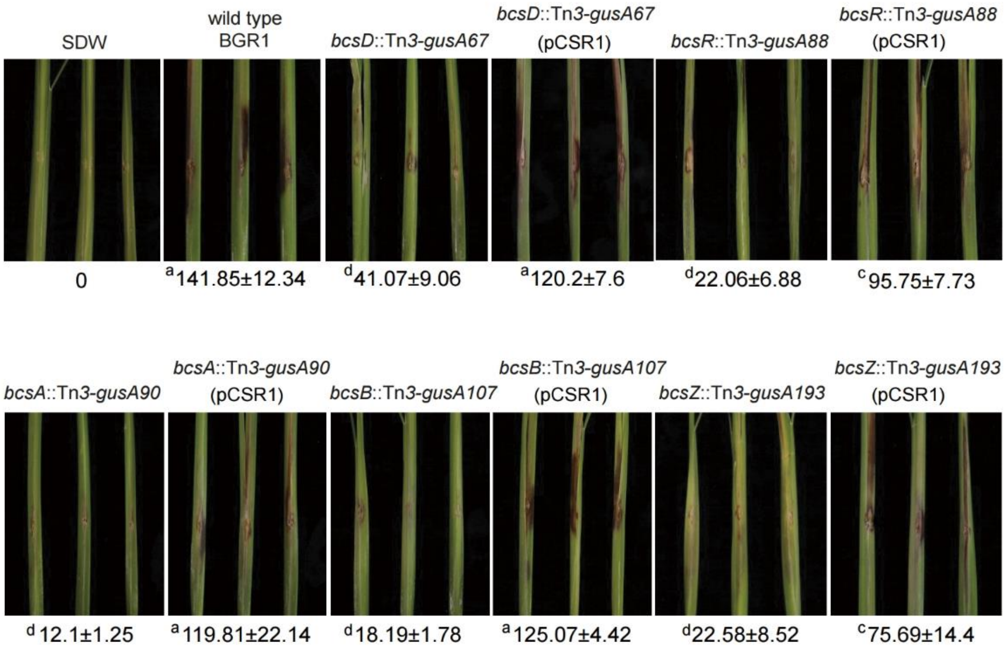
Figure S1. Virulence and colonization assays of all mutated and complemented strains for seven putative cellulose biosynthesis regulatory genes in pSCR1..……………….………….Page 2–3

Table S1. Strains and plasmids used in this study……………………………………..…Page 4–5

Table S2. Identified GGDEF/EAL domain encoding genes in *B. glumae* …………….……..Page 6–7

Supplemental references for Table S1……….……………………………………………..Page 7–8

A


SDW
wild type BGR1
bcsD::Tn*3-gusA67*
bcsD::Tn*3-gusA67* (pCSR1)
bcsR::Tn*3-gusA88*
bcsR::Tn*3-gusA88* (pCSR1)
0
a 141.85±12.34
d 41.07±9.06
a 120.2±7.6
d 22.06±6.88
c 95.75±7.73
bcsA::Tn*3-gusA90*
bcsA::Tn*3-gusA90* (pCSR1)
bcsB::Tn*3-gusA107*
bcsB::Tn*3-gusA107* (pCSR1)
bcsZ::Tn*3-gusA193*
bcsZ::Tn*3-gusA193* (pCSR1)
d 12.1±1.25
a 119.81±22.14
d 18.19±1.78
a 125.07±4.42
d 22.58±8.52
c 75.69±14.4

B

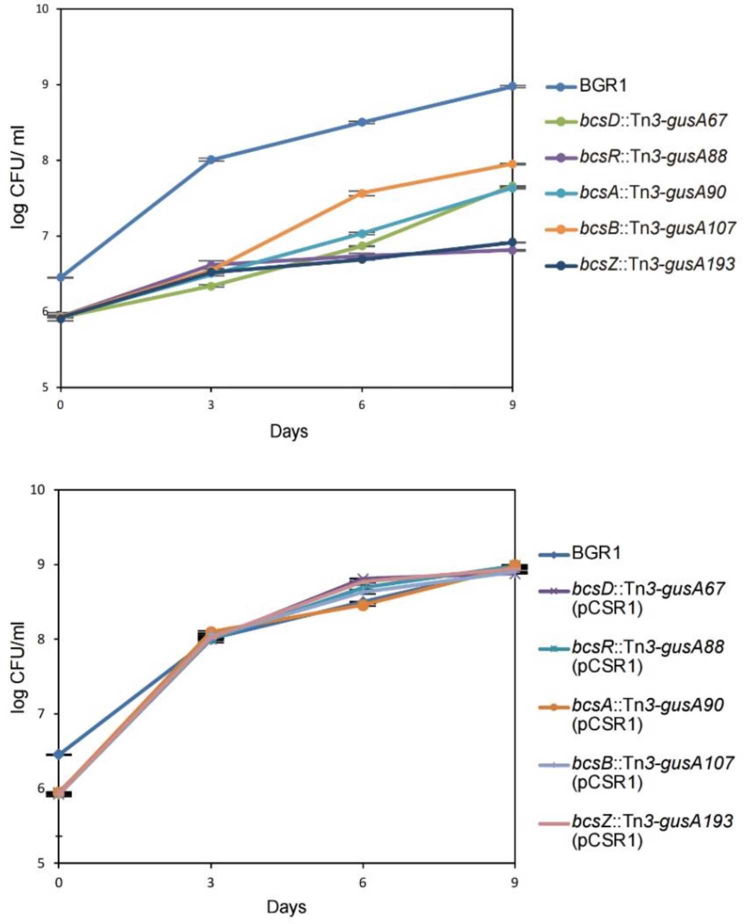

10
9
8
7
6
5
log CFU/ ml
0
3
6
9
Days
BGR1
bcsD::Tn*3-gusA67*
bcsR::Tn*3-gusA88*
bcsA::Tn*3-gusA90*
bcsB::Tn*3-gusA107*
bcsZ::Tn*3-gusA193*
10
9
8
7
6
5
log CFU/ml
0
3
6
9
Days
BGR1
bcsD::Tn*3-gusA67* (pCSR1)
bcsR::Tn*3-gusA88* (pCSR1)
bcsA::Tn*3-gusA90* (pCSR1)
bcsB::Tn*3-gusA107* (pCSR1)
bcsZ::Tn*3-gusA193* (pCSR1)

Figure S1. Virulence assay of non-pellicle forming cellulose mutant strains. **(A)** The pellicle defective mutants with Tn*3-gusA* insertion in putative cellulose biosynthetic genes, *bcsD*, *R*, *A*, *B*, and *Z*, exhibited no serious symptoms in rice sheaths compared to wild-type strain BGR1. The complemented strains exhibited full recovery of virulence. The superscripts (a, b, c, and d) on the mean values represent a significant difference ($p < 0.05$) based on ANOVA/Tukey's correction for multiple comparisons in virulence scores in pixels among treatments. Values are presented as the mean ± standard deviation. **(B)** Changes in population of wild type, non-pellicle producing cellulose mutants, and complementation strains in rice sheaths. The colonization ability of the cellulose mutants with restored pellicle production was recovered to the level of wild-type strain BGR1.

Table S1. Strains and plasmids used in this study.

Bacterial strain or plasmid	Genotype	Source or reference
Strains		
Burkholderia glumae		
BGR1	Wild-type, Rif^{R}	(Jeong et al., 2003)
BGS2	BGR1, *tofI*::Ω	(Kim et al., 2004)
BGS9	BGR1, *qsmR*::Ω	(Kim et al., 2007)
BGA90	BGR1, *bcsA*::Tn*3-gusA90*	This study
BGB107	BGR1, *bcsB*::Tn*3-gusA107*	This study
BGC45	BGR1, *bcsC*::Tn*3-gusA45*	This study
BGD67	BGR1, *bcsD*::Tn*3-gusA67*	This study
BGZ193	BGR1, *bcsZ*::Tn*3-gusA193*	This study
BGQ6	BGR1, *bcsQ*::Tn*3-gusA6*	This study
BGR88	BGR1, *bcsR*::Tn*3-gusA88*	This study
BGF42	BGR1, *flhA*::Tn*3-gusA42*	(Kim et al., 2007)
BGF43	BGR1, *cheB*::Tn*3-gusA43*	(Kim et al., 2007)
BGF45	BGR1, *fliA*::Tn*3-gusA45*	(Kim et al., 2007)
BGF48	BGR1, *cheZ*::Tn*3-gusA48*	(Kim et al., 2007)
Escherichia coli		
DH5α	F^{-} Φ80d*lacZ*Δ*M15*Δ(*lacZYA-argF*) *U169* endA1 *recA1 hsd1 hsdR17*(r$_{k}^{-}$m$_{k}^{+}$) *deoR thi-1supE44*λ^{-} *gyrA96 relA1*	Gibco BRL
S17-1	Tra^{+}, *recA*, Sp^{R}	(Simon et al., 1983)
C2110	*polA*, Nal^{R}	(Stachel et al., 1985)

Plasmids		
pSRKKm	pBBR1MCS-2-derived broad-host-range expression vector containing lac promoter and lacI^q and lacZα$^+$, Km^R	(Khan et al., 2008)
pBluescript II SK(+)	pUC derivative, Amp^R	Stratagene
pLysS	Encodes T7 lysozyme gene, Cm^R	Novagen
pSShe	Cm^R	(Stachel et al., 1985)
pHoKmGus	Promoterless *β*-glucuronidase gene, Km^R, Amp^R *tnpA*	(Bonas et al., 1989)
pRK2013	Tra$^+$, ColE1 replicon, Km^R	(Figurski and Helinski, 1979)
pLAFR3	Tra$^-$, Mob$^+$, RK2 replicon, Tet^R	(Staskawicz et al., 1987)
pJW110	pSRKKm carrying P_{lac}-*pleD*, Km^R	(Xu et al., 2013)
pCOK76	Plasmid vector carrying *lacI^q*, P*lac*, *lacZα*, and *pelI*, Km^R	This study
pCSR1	Plasmid harboring *bcsA*, *B*, *C*, *D*, *Z*, and *yhjQ*	This study
pBGF2	23 kb DNA fragment harboring *fliA*, *flhA*, *CheZ*, and *CheB* from BGR1 cloned into pLAFR3	(Kim et al., 2007)

Table S2. Identified GGDEF/EAL domain encoding genes in *B. glumae.*

Locus ID*	Domain	Annotation
BGLU_RS01045	REC/GGEEF	Diguanylate cyclase 6
BGLU_RS05070	PAC/GGDEF/EAL	Diguanylate cyclase/phosphodiesterase (GGDEF & EAL domains) with PAS/PAC sensor(s)
BGLU_RS05175	EAL	Hypothetical protein BDAG_01993
BGLU_RS06570	GGDEF/EAL	Diguanylate cyclase/phosphodiesterase with PAS/PAC sensor (frame shifted)
BGLU_RS09190	GGDEF/EAL	Diguanylate cyclase 3
BGLU_RS12055	EAL/HDOD	Diguanylate phosphodiesterase2/*Burkholderia cenocepacia* pBCA055
BGLU_RS13960	GGDEF/EAL	Hypothetical protein BMASAVP1 A1055
BGLU_RS14170	GGDEF/EAL	Diguanylate cyclase/phosphodiesterase 2
BGLU_RS17000	EAL	Cyclic diguanylate phosphodiesterase
BGLU_RS17085	GGDEF/EAL	Diguanylate cyclase/phosphodiesterase
BGLU_RS17210	GGDEF	Porin, Gram-negative type
BGLU_RS18985	GGDEF	Hypothetical protein BB4664
BGLU_RS21375	GGDEF/EAL	Glycogen debranching enzyme Glgx 4
BGLU_RS21385	PAS/GGDEF	Diguanylate cyclase with PAS/PAC sensor (*pelI*)
BGLU_RS22710	GGDEF/EAL	Thioester reductase
BGLU_RS24205	GGDEF	Allophanate hydrolase subunit
BGLU_RS25010	GGDEF/EAL	Diguanylate cyclase/phosphodiesterase 4

BGLU_RS25390	GGDEF	Glycogen debranching enzyme Glgx2
BGLU_RS27365	EAL	Diguanylate phosphodiesterase 4
BGLU_RS28165	EAL	Diguanylate phosphodiesterase 3
BGLU_RS28585	GGDEF/EAL	Diguanylate cyclase/phosphodiesterase 3
BGLU_RS29240	HD-GYP	Metal-dependent phosphohydrolase
BGLU_RS17205	HD	Metal-dependent phosphohydrolase

*Locus IDs were obtained from GenBank.

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