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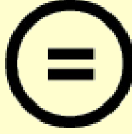

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농학박사학위논문

*Burkholderia glumae*의 Quorum Sensing과 RpoS에 의한 Universal Stress Protein 유전자의 발현조절

Regulation of Universal Stress Protein Genes by Quorum Sensing and RpoS in *Burkholderia glumae*

2013년 2월

서울대학교 대학원

농생명공학부 식물미생물학전공

김 홍 섭

Regulation of Universal Stress Protein Genes by Quorum Sensing and RpoS in *Burkholderia glumae*

A dissertation submitted in partial
fulfillment of the requirement for
the degree of

DOCTOR OF PHILOSOPHY

to the Faculty of
Department of Agricultural Biotechnology

at

SEOUL NATIONAL UNIVERSITY

by

Hongsup Kim

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농학박사학위논문

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A THESIS FOR THE DEGREE OF DOCTOR OF PHILOSOPHY

Regulation of Universal Stress Protein Genes by Quorum Sensing and RpoS in *Burkholderia glumae*

UNDER THE DIRECTION OF DR. INGYU HWANG

SUBMITTED TO THE FACULTY OF THE GRADUATE SCHOOL
OF SEOUL NATIONAL UNIVERSITY

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Regulation of Universal Stress Protein Genes by Quorum Sensing and RpoS in *Burkholderia glumae*

Hongsup Kim

ABSTRACT

Universal stress proteins (Usps) are ubiquitous in bacteria, fungi, flies, and plants, and are highly expressed at high cell densities and in response to stasis. They play important roles in defense against stresses, thereby contributing to the survival of bacteria in the environment. Because *usp* genes are highly expressed at a high cell density, quorum sensing (QS) may be involved in *usp* gene regulation. QS is a coordinated gene-regulation system that controls social behavior of bacteria such as virulence, biofilm formation, and toxin production in response to cell density. Rice panicle blight is caused by *Burkholderia glumae* and can spread dramatically in rice-growing areas. The bacterium produces a phytotoxin called toxoflavin at 37℃ in a QS-dependent manner and possesses 11 *usp* genes. The facts that QS and an alternative sigma factor RpoS control *usp* gene expression are unique features of *usp* genes in *B*.

glumae. This is the first evidence that the expression of *usp* genes depends on cell density and growth phase. Compared to the diverse roles of Usps in *Escherichia coli*, Usps in *B*. *glumae* are dedicated to heat shock stress.

KEY WORDS: *usp* genes, Quorum sensing, *Burkholderia glumae*, Heat Shock Stress

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CONTENTS

Page

ABSTRACT...i
CONTENTS...iii
LIST OF TABLES...v
LIST OF FIGURES...vi

Regulation of Universal Stress Protein Genes by Quorum Sensing and RpoS in *Burkholderia glumae*

ABSTRACT...2
INTRODUCTION...3
MATERIALS AND METHODS...7
I. Bacterial strains and culture conditions...7
II. Protein sample preparation for two-deimensional electrophoresis (2-DE)...7
III. 2-DE and tandem mass spectrometry (MS/MS) analysis...8
IV. Multiple-sequence alignment and phylogenetic analysis...10
V. DNA construction and mutagenesis...10
VI. Reverse transcription (RT)-PCR analysis...12
VII. Overexpression and purification of *B. glumae* QsmR and RpoS...12
VIII. Gel mobility shift assay...13
IX. β-Glucuronidase assay...15
X. Survival tests under heat shock stress...15
XI. Survival under cold shock stress...16
XII. Sensitivity to oxidative stress...16
XIII. Motility assay...16
XIV. UV sensitivity...17
XV. Sensitivity to ethanol, acid, and base...17
XVI. Long-term survival in the stationary phase...17
XVII. Sensitivity to mitomycin C and osmotic stress...17
XVIII. Plant Inoculation...18
RESULTS...19

I. Identification of QS-dependent *usp* genes in *B. glumae*……………………19
II. Classification of Usps and their phylogenetic relationships………………19
III. Genomic organization of the *usp* genes of *B. glumae*……………………..21
IV. Phenotypes of *usp* mutants under stress conditions………………………22
V. Expression of *usp* genes is regulated by QsmR…………………………….23
VI. Expression of *usp1* and *usp2* is dependent on RpoS……………………..24
VII. Expression of *usp1* is upregulated by a temperature shift……………….25
VIII. Mutations of *usp1* and *usp2* retain full virulence……………………….26
DISCUSSION……………………………………………………………………..27
LITERATURE CITED…………………………………………………….. 32
ABSTRACT IN KOREAN……………………………………………….77
ACKNOWLEDGEMENT…………………………………………………79

LIST OF TABLES

Page

Table 1. Strains and plasmids used in this study……………………………39
Table 2. Primers used for RT-PCR…………………………………………44
Table 3. Primers used for gel shift assays………………………………..45
Table 4. Identification of Usp2 under the control of QS in *B. glumae*………..46
Table 5. Annotation of *B. glumae* genes encoding Usps………………………47
Table 6. Identity among Usps in *B. glumae*..48
Table 7. Expression of *usp* genes in *B. glumae*………………………………..49

LIST OF FIGURES

Page

Fig. 1. Two-dimensional gel analysis of *B. glumae* cell extracts…………….....50
Fig. 2. Phylogenetic relationships among Usps of *E. coli* and
B. glumae and conserved ATP-binding motifs………………………..52
Fig. 3. Prediction of serine and threonine residues in *B. glumae* Usps………..54
Fig. 4. Genetic organization of *usp* genes in *B. glumae*………………………56
Fig. 5. Survival of *usp* mutants after exposure to heat shock
in the stationary phase………………………………………………...58
Fig. 6. Comparison of the stress sensitivity of the *usp1* and *usp2* mutants……60
Fig. 7. Swimming and swarming motilities of *usp* mutants in *B. glumae*…….62
Fig. 8. Gel mobility shift assay of the *usp1* and *usp2* regulatory regions
using QsmR-His, His-RpoS, and *E. coli* RNA core enzyme…………64
Fig. 9. Gel mobility shift assay using purified QsmR-His and a DNA fragment
that contained the *B. glumae usp3-4-5*, *6*, *7*, *8-9*, *10*, and *11* regulatory
regions…………………………………………………………………..66
Fig. 10. Expression of the *usp1* and *usp2* genes at different growth phases
and under different genetic backgrounds……………………………..68
Fig. 11. Expression of *rpoS* is independent of QS……………………………70
Fig. 12. Expression of the *usp1* and *usp2* genes under a temperature shift……72
Fig. 13. Pathogenicity of the *usp1* and *usp2* mutants…………………………74

Regulation of Universal Stress Protein Genes by Quorum Sensing and RpoS in *Burkholderia glumae*

ABSTRACT

Burkholderia glumae possesses a quorum sensing (QS) system mediated by *N*-octanoyl-homoserine lactone (C_8-HSL) and its cognate receptor TofR. TofR/C_8-HSL regulates the expression of a transcriptional regulator, *qsmR*. We identified one of the universal stress proteins (Usps), Usp2, from a genome-wide analysis of QS-dependent proteomes of *B. glumae*. In the whole genome of *B. glumae* BGR1, 11 *usp* genes (*usp1* to *usp11*) were identified. Among the stress conditions tested, *usp1* and *usp2* mutants died 1 h after heat shock stress, whereas the other *usp* mutants and the wild-type strain survived for more than 3 h at 45℃. The expression of all *usp* genes was positively regulated by QS, directly by QsmR. In addition, the expression of *usp1* and *usp2* was dependent on RpoS in the stationary phase, as confirmed by the direct binding of RpoS-RNA holoenzyme to the promoter regions of the *usp1* and *usp2* genes. The expression of *usp1* was upregulated upon a temperature shift from 37℃ to either 28℃ or 45℃, whereas the expression of *usp2* was independent of temperature stress. This indicates that the regulation of *usp1* and *usp2* expression is different from what is known about *Escherichia coli*. Compared to the diverse roles of Usps in *E. coli*, Usps in *B. glumae* are dedicated to heat shock stress.

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INTRODUCTION

Bacteria possess internal and external protection mechanisms to overcome environmental stresses such as nutrient starvation, temperature stress, oxidative stress, and toxic agents. One of the stress-responsive genes against various deleterious stresses is the universal stress protein (*usp*) gene (Nystrom *et al*., 1992). The *usp* genes were first reported for *Escherichia coli* (Nystrom *et al*., 1992) and are found in archaea, fungi, flies, and plants (Kvint *et al*., 2003). In *E. coli*, six bona fide *usp* genes exist: *uspA* and *uspC* to *uspG* (Kvint *et al*., 2003). These are divided into four classes based on their amino acid sequence similarities (Kvint *et al*., 2003). UspA, UspC, and UspD belong to class I, and UspF and UspG are members of class II. UspE possesses two domains, E1 and E2, which were previously classified as class III and class IV, respectively (Kvint *et al*., 2003). In *E. coli*, three isoforms of UspA have been identified *in vivo*, and two of them are phosphorylated on serine and threonine residues in response to stasis (Freestone *et al*., 1997). UspG is dimeric and possesses autophosphorylation and autoadenylation activities (Weber *et al*., 2006). Although there have been many reports regarding the roles of Usps in defending against diverse stresses in bacteria, how Usps function biochemically is not known.

The expression of *usp* genes in *E. coli* is regulated primarily at the transcription level from a σ^{70}-dependent promoter (Nystrom *et al*., 1994). The alarmone guanosine tetraphosphate (ppGpp), which is important for the regulation of many stationary-phase-induced genes, coordinately regulates four *usp* genes (*uspA*, *uspC*, *uspD*, and *uspE*) in *E. coli* (Kvint *et al*., 2000; Kvint *et al*., 2003). The expression of *uspA* is negatively regulated by FadR, an activator or a repressor of fatty acid biosynthesis and degradation genes, respectively (Farewell *et al*., 1996). The *uspA*, *uspC*, *uspD*, and *uspE* genes are highly expressed in the stationary phase of the carboxy domain deletion mutant of FtsK, a RecA-like double-stranded (dsDNA) translocase (Diez *et al*., 1997). However, the induction of the UspA protein is independent of RpoS (σ^{S}) (Nystrom *et al*., 1992). In *E. coli*, UspA is involved in oxidative stress defense, and UspD is involved in both oxidative stress defense and iron scavenging (Nachin *et al*., 2005). UspC, UspF, and UspG are involved mainly in motility and adhesion (Nachin *et al*., 2005). In *Pseudomonas aeruginosa*, Usps are essential for survival under conditions of anaerobic energy stress and are required for pyruvate fermentation (Boes *et al*., 2006; Schreiber *et al*., 2006).

Considering that *usp* genes of *E. coli* are highly expressed in the stationary phase, *usp* gene expression might depend on bacterial cell density. The regulation of bacterial gene expression that is dependent on cell density is called quorum sensing (QS). QS is an intercellular signaling circuit that

regulates sets of genes involved in certain social behaviors of bacteria (Waters *et al*., 2005). QS plays important roles in physiological changes, including biofilm formation, motility, protein secretion, virulence, antibiotic production, and protection against stress defense in bacteria (Chun *et al*., 2009; Goo *et al*., 2010; Hassett *et al*., 1999; Kim *et al*., 2007; von Bodman *et al*., 2003; Water *et al*., 2006). However, whether QS regulates the expression of *usp* genes in bacteria is not known.

In the present study, we examined QS-dependent biological phenomena of *Burkholderia glumae*, which is the causal agent of rice panicle blight (also called bacterial rice grain rot). The bacterium produces a phytotoxin called toxoflavin at the optimum growth temperature (37℃) in a QS-dependent manner and infects rice panicles during the flowering stage (Kim *et al*., 2004). Rice panicle blight has recently become widespread in most rice-growing areas and can be a serious threat, particularly when hot and humid weather conditions persist during the flowering stage (Jeong *et al*., 2003). The bacterium possesses a LuxR-LuxI-type QS system. TofI is an *N*-acyl homoserine lactone synthase for the synthesis of *N*-octanoyl homoserine lactone (C_8-HSL) that is recognized by a cognate receptor, TofR (Kim *et al*., 2004). A complex of TofR and C_8-HSL activates the expression of *qsmR*, an IclR-type transcriptional regulator (Kim *et al*., 2007). QsmR activates the expression of flagellum genes and type II protein secretion genes (Goo *et al*., 2010; Kim *et al*., 2007).

From genome-wide proteome analysis of the bacterium, we found that one of the Usps is under the control of QS. Based on this finding, we decided to characterize all the *usp* genes in the whole genome of *B. glumae* BGR1. We identified 11 *usp* genes that are phylogenetically distinct from the 6 *usp* genes in *E. coli*. Unlike *usp* gene regulation in *E. coli*, the expression of *usp* genes in *B. glumae* depends on QS and RpoS. We show that Usp1 and Usp2 play important roles in the survival of *B. glumae* under conditions of heat shock stress.

The objectives of this study were;

1) How *usp* genes are regulated and what the functional roles of Usps are in *B. glumae* BGR1.

2) Biochemically, how regulator preteins bind to their target promoter regions for *usp* genes.

MATERIALS AND METHODS

I. Bacterial strains and growth conditions

The bacterial strains and plasmids used in this work are shown in Table 1. *B. glumae* BGR1 strains and all *E. coli* strains were grown in LB medium (1% [wt/vol] tryptone and 0.5% [wt/vol] of yeast extract [pH 7.0]; USB Corp., Cleveland, OH) at 37℃ or 28℃. Antibiotics were used at the following concentrations: ampicillin at 100 μg ml^{-1}, chloramphenicol at 20 μg ml^{-1}, kanamycin at 50 μg ml^{-1}, nalidixic acid at 20 μg ml^{-1}, rifampicin at 100 μg ml^{-1}, spectinomycin at 100 μg ml^{-1}, tetracycline at 10 μg ml^{-1}, and gentamycin at 20 μg ml^{-1}.

II. Protein sample preparation for two-dimensional electrophoresis (2-DE)

Cultures of *B. glumae* strains grown overnight in LB medium were diluted 1:100 in LB medium and grown at 37℃ with shaking for 24 h in the stationary phase. Next, 1 μM C_8-HSL was added to a culture of BGS2 (*tofI*::Ω) cells when necessary. Cells were harvested in the stationary phase by centrifugation and washed three times with 20 mM Tris-HCl (pH 7.4), suspended in 20 mM Tris-HCl (pH 7.4), and lysed by sonication with a VCX-400 sonicator (Sonics & Materials, Newton, CT). Cellular proteins were precipitated with 10% (wt/vol) trichloroacetic acid, followed by centrifugation

at 12,000 × *g* for 30 min at 4°C, and the protein concentration of each sample was determined by using the Bradford assay and bovine serum albumin (BSA) as a standard (Bradford MM. 1976).

III. 2-DE and tandem mass spectrometry (MS/MS) analysis

A total of 100 μg of each protein sample was resuspended in 450 μL rehydration solution containing 8 M urea, 2% (wt/vol) CHAPS {3-[(3-cholamidopropyl)-demethylammonio]-1-propanesulfonate}, 2% (vol/vol) immobilized pH gradient (IPG) buffer (GE Healthcare), 100 mM dithiothreitol (DTT), and 0.002% (wt/vol) bromophenol blue. The proteins were focused in the first dimension by using 24-cm-long IPG strips (GE Healthcare) of pH 4 to 7. Isoelectric focusing was performed using an Ettan IPGphor (GE Healthcare) at a constant temperature of 20°C with a total of 74,500 V/h as follows: 80 V for 1 h; 500 V for 1 h; 1,000 V for 1 h; and 8,000 V up to 74,500 V/h. The strips were equilibrated before the second gel was run, as described previously (Gorg *et al*., 1995). For sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE), we used 12.5% T-acrylamide-bisacrylamide (37.5:1) gels to separate proteins in the 1- and 100-kDa ranges using an Ettan DALTsix Large Vertical Electrophoresis System (GE Healthcare). After electrophoresis, proteins were visualized using a silver staining kit (GE Healthcare) as recommended by the manufacturer. 2-DE was

performed several times for each strain.

2-DE gel image analysis was performed by visual inspection or by using PDQuestTM 2-D Analysis V 8.0 software (Bio-Rad). After optical density calibration, spot volumes were normalized according to the total spot volume in each gel. The analysis of protein spots was independently repeated at least three times. Electrospray ionization (ESI)-tandem mass spectrometry (MS/MS) of the peptides generated by in-gel digestion was performed by nano-ESI on a Q-TOF2 mass spectrometer (Micromass, Manchester, United Kingdom). The data were processed by using the MassLynx, versoin 3.5, Windows NT system (Micromass), and *de novo* sequencing from the fragmentation spectra of the peptides was performed by using PepSeq (Micromass). To assign a positive protein identification, parameters of up to 1 missed cleavage, fixed modifications of carbamidomethyl (C), a peptide tolerance ± 0.6 Da, and a peptide charge of 2+ or 3+ were used. The resulting sequences were searched against a FASTA format of the translated open reading frames (ORFs) in the *B. glumae* BGR1 genomic database at the KROPBASE website (http://kropbase.snu.ac.kr). Search results were accepted when the *de novo* peptide sequencing data comprised at least eight matched amino acids, and the matched results were below the E value inclusion threshold (E=0.05). A homology search of each putative ORF with proteins present in the NCBI database was performed by using the BLAST program (Altschul *et al*., 1990).

IV. Multiple-sequence alignment and phylogenetic analysis

All *usp* gene sequences were retrieved from the NCBI website (http://www.ncbi.nlm.nih.gov/) through similarity searches performed by using BLASTP (http://ncbi.nlm.nih.gov/BLAST). The retrieved sequences from *E. coli* and *B. glumae* were aligned by using the "create alignment" function in the CLC main workbench 5.6 program (CLCbio). The evolutionary distances between the sequences were computed by using an algorithm described previously by Jukes and Cantor (Jukes *et al.*, 1969). Phylogenetic tree calculation was performed by using the neighbor-joining method (Saitou *et al.*, 1987) in the CLC Main Workbench 5.6 program. The statistical significances of the phylogenies were tested by using bootstrap analysis (Felsenstein *et al.*, 1985), with each bootstrap value reflecting the confidence of each branch. Determinations of identity among Usps in *B. glumae* were performed by using the LALIGN program (Huang *et al.*, 1991).

V. DNA construction and mutagenesis

General and standard techniques were used for DNA manipulations, cloning, restriction digestions, and agarose gel electrophoresis (Sambrook *et al.*, 1989). Mutagenesis of genes with Tn*3-gusA* was performed, and the insertion site and orientation of Tn*3-gusA* in each mutant were determined as described previously (Kim *et al.*, 2004). The mutagenized cosmids that carried Tn*3-gusA*

insertions were introduced individually by conjugation and then marker exchanged into wild-type strain BGR1 and stranis BGS2 (*tofI*::Ω) and BGS9 (*qsmR*::Ω) as described previously (Kim *et al*., 2004). To construct double mutants of *usp1* or *usp2* and *rpoS*, the gentamycin cassette from pBSGm was inserted into the unique AfeI site in the 4.0-kb DNA fragment containing *rpoS* in pRPOS (Table 1). The resulting plasmid, pRPOS1, was introduced into strains BGQ8 (*usp1*::Tn*3-gusA20*), BGSQ8 (*tofI*::Ω *usp1*::Tn*3-gusA20*), and BGH8 (*qsmR*::Ω *usp1*::Tn*3-gusA20*) to construct double mutations of *usp1* and *rpoS*, or into strains BGQ9 (*usp2*::Tn*3-gusA104*), BGSQ9 (*tofI*::Ω *usp2*::Tn*3-gusA104*), and BGH9 (*qsmR*::Ω *usp2*::Tn*3-gusA104*) to construct double mutations of *usp2* and *rpoS* by marker exchange. To construct double mutants of *usp1* and *usp2*, the spectinomycin cassette from pHP45Ω was inserted into the unique EcoNI site in the 5.4-kb DNA fragment carrying *usp1* in pBGQ8 (Table 1). The resulting plasmid, pUSP1, was introduced into BGQ9 (*usp2*::Tn*3-gusA104*) cells by marker exchange. All marker exchanges were confirmed by Southern hybridization analysis using each cosmid as a probe.

For *usp1* complementation, we amplified a 1,046-bp PCR product carrying a putative promoter region and structural gene using primers usp1C-F (5′-AGCAGCATCCGCAGCGTGGT-3`) and usp1C-R (5`-CGATCGATCTGCAGCCCTGA-3′). The PCR product was subcloned into the SmaI site of pBluescriptII SK (+), followed by cloning into pRK415 as an

XbaI- and -KpnI fragment (Table 1). The resulting plasmid, pHS1, was introduced into BGQ8 (*usp1*::Tn*3*-*gusA20*) by conjugation. For *usp2* complementation, the 1,649-bp EcoRI fragment from pBGQ9 was subcloned into pRK415, resulting in pHS2, followed by introduction into BGQ9 (*usp2*::Tn*3*-*gusA104*) cells by conjugation.

VI. Reverse transcription (RT)-PCR analysis

Total RNA was isolated by using an RNeasy mini kit (Qiagen) according to the manufacturer's protocol. Isolated RNA was treated with DNaseI (Qiagen) for 30 min at 37℃. A total of 1 μg RNA was reverse transcribed into cDNA by using Moloney murine leukemia (M-MLV) reverse transcriptase (Promega, Madison, WI) for 1 h at 42℃. PCR was performed by using a PTC-200 Thermo Cycler (MJ Research, Waltham, MA) under the following conditions: 96℃ for 5 min, followed by 35 cycles of 96℃ for 1 min, 55℃ for 1 min, and 72℃ for 2 min. The primers used for each reverse transdription (RT)-PCR reaction are listed in Table 2.

VII. Overexpression and purification of *B. glumae* QsmR and RpoS

QsmR was overexpressed and purified as described previously (Kim *et al*., 2007). To overexpress RpoS in *E. coli*, the coding region of *rpoS* was amplified by using pRPOS as the template DNA and the oligonucleotide

primers RPOS-F (5′-CGAGACGCATATGCCGAAAT-3′) and RPOS-R (5′-ATCGGATCCTTACAGAACGG-3′), which introduced unique NdeI and BamHI sites at the ends of the PCR product, and the amplified product was cloned into the corresponding sites of pET14b (Invitrogen), resulting in pRPOS-His (Table 1). His-RpoS was overexpressed in *E. coli* strain BL21 (DE3), which carries pLysS, according to instructions provided by the manufacturer (Novagen). Soluble His-RpoS was purified in a buffer containing 10 mM Tris-HCl (pH 8.0), 0.1 mM EDTA, 1 mM DTT, 5% glycerol, and 200 mM NaCl (Lee *et al*., 2001), using a Ni-nitrilotriacetic acid (NTA) spin column according to the manufacturer`s instructions (Qiagen).

VIII. Gel mobility shift assay

The 340-bp regions upstream of the *usp* genes were amplified by PCR using primers for each *usp* gene (Table 3). The fragments were eluted from an agarose gel and labeled with biotin for chemiluminescence by using a Lightshift chemiluminescent electrophoretic mobility shift assay (EMSA) kit (Pierce). For competitor DNA, we used the 242-bp upstream region of *katE*, which was amplified by using KEN1 and KEN2 primers as described previously (Kim *et al*., 2007). Purified QsmR-His (250 nM) was incubated with 1 nM biotin-labeled DNA in binding buffer [10 mM Tris-HCl (pH 7.5), 10 mM NaCl, 100 mM NaCl, 0.5 mM EDTA, 1 mM DTT, 5% (vol/vol) glycerol,

and 50 ng μl^{-1} poly (dI·dC)] for 15 min at 28°C. For the competitor DNA, a 27-fold molar excess of unlabeled target DNA was added to the reaction mixture along with the extract, before the labeled DNA target was added. The mixtures were size fractionated on a nondenaturing 4% polyacrylamide gel, followed by drying and transfer onto nitrocellulose membranes. Signals were detected by streptavidin-horseradish peroxidase (HRP) chemiluminescence for biotin-labeled probes according to the manufacturer`s instructions (Pierce).

To perform an EMSA with RpoS and RpoS-RNA holoenzyme, *E. coli* RNA polymerase was purchased from Epicentre Technologies. EMSAs were conducted according to methods reported in a previous study (Lee *et al*., 2001), with some modifications; briefly, 50 nM or 100 nM core RNA polymerase was mixed with 500 nM His-RpoS in a 10-μl reaction mixture with 1× buffer A [50 mM Tris-HCl at pH 7.9, 200 mM potassium glutamate, 3 mM $MgCl_2$, 0.1 mM EDTA, 1 mM DTT, 100 $\mu g\ ml^{-1}$ BSA, 6 ng μl^{-1} poly (di·dC)], incubated at 28°C for 10 min to allow RNA holoenzyme formation, and then added to 1 nM biotin-labeled DNA and incubated for 25 min on ice. For heparin challenge experiments, 0.5 μl 1 mg ml^{-1} heparin was added for an additional 10 min. All samples were run on 4% polyacrylamide gels at 100 V with cold 1× Tris-borate-EDTA in a cold room, and then transferred onto nitrocellulose membranes for detection by streptavidin-HRP chemiluminescence for biotin-labeled probes. The images were visualized and quantified using Chemi

Doc XRS+ with Image Lab Software (Bio-Rad).

IX. β-Glucuronidase assays

β-Glucuronidase (GUS) activity assays were performed at least three times, as described previously (Jefferson *et al.*, 1987), with modifications. The expression of *usp* genes was evaluated under three different conditions. All of the BGR1 derivatives were grown in LB medium at 37℃ for 12 h with shaking and then shifted to a temperature of either 28℃ for 4 h or 45℃ for 15 min with shaking for cold and heat stress conditions. C_8-HSL was added at a final concentration of 1 μM when the cells were subcultured. The bacteria were collected by centrifugation, resuspended in GUS extraction buffer, and lysed by sonication using a VCX-400 sonicator (Sonics & Materials). The extract was used in a β-glucuronidase enzyme assay with 4-methylumbelliferyl glucuronidase as the substrate. The fluorescence was measured at 365-nm excitation and 460-nm emission in a Hoefer DQ300 fluorometer (Hoefer Scientific Instruments, San Francisco, CA). One unit of β-glucuronidase activity was defined as the amount of enzyme required to release 1 nmol 4-methylumbelliferone per bacterium per min.

X. Survival tests under heat shock stress

All *B. glumae* BGR1 derivatives were grown at 37℃ for 12 h in LB

medium, and then shifted to 45℃ with shaking. Samples were taken periodically to measure colony forming units on LB plates at 37℃ after serial dilution.

XI. Survival under cold shock stress

B. glumae derivatives were grown to the stationary phase in LB medium at 37℃ for 12 h with shaking and transferred to 28℃ for 7 days, and then viable cells were counted daily.

XII. Sensitivity to oxidative stress

To assess survival under conditions of hydrogen peroxide and t-BOOH stress, *B. glumae* derivatives were grown to the stationary phase in LB medium at 37℃ for 12 h with shaking. Then the cells were exposed to a 10-fold diluted series of hydrogen peroxide (0 to 10 mM) for 0, 10, and 20 min at 37℃ or t-BOOH (0 to 0.03%) for 4 h at 37℃.

XIII. Motility assay

All *B. glumae* derivatives were grown overnight in LB medium at 37℃. All the motility and adhesion assays were performed at 37℃ on LB agar plates that contained 0.7% or 0.3% Bacto agar (Difco), respectively, as described previously (Kim *et al*., 2007).

XIV. UV sensitivity

All *B. glumae* derivatives grown overnight in LB medium at 37℃ were plated onto LB agar plates after serial dilution. Then the plates were irradiated with UV light at different intensities (0 to 22.5 mJ/cm^2) using the transilluminator (Spectroline) at a wavelength of 312 nm. The survivors were counted as colony-forming units after overnight incubation at 37℃.

XV. Sensitivity to ethanol, acid, and base

Overnight cultures of *B. glumae* derivatives were exposed to 4% EtOH for 4 h at 37℃, or to pH 4 or pH 10 for 0.5 and 4 h at 37℃, and then viable cells were counted.

XVI. Long-term survival in the stationary phase

All *B. glumae* derivatives were grown in LB medium aerobically at 37℃ for several days with shaking. Viable cell counts were counted daily up to 8 days.

XVII. Sensitivity to mitomycin C and osmotic stress

All *B. glumae* derivatives were exposed to a twofold diluted series of mitomycin C (1 $\mu g\ ml^{-1}$) or 1 M NaCl for 0 and 4 h at 37℃, and viable cells were counted.

XVIII. Plant Inoculation

Rice plants (*Oryzae sativa* cv. Milyang 23) were grown in a greenhouse and inoculated in the flowering stage with *B. glumae* at approximately 1×10^8 CFU ml^{-1} as described previously (Kim *et al.*, 2004). The disease in the rice plants was evaluated 7 days after inoculation, and the disease index was determined as described by Iiyama et al. (Iiyama *et al.*, 1995), using the following scale: 0=healthy panicle, 1=panicle 0–20% discolored, 2=panicle 20–40% discolored, 3= panicle 40–60% discolored, 4= panicle 60–80% discolored, and 5=panicle 80–100% discolored. Disease degree = Σ(number of samples per score×score)/the total number of panicles. Pathogenicity assays were repeated three times with three replications.

RESULTS

I. Identification of QS-dependent *usp* genes in *B. glumae*

We identified one of the Usps, Usp2, from the genome-wide analysis of QS-dependent proteomes of *B. glumae* (Fig. 1). The estimated molecular mass and pI of Usp2 were 15.2 kDa and 5.17, respectively (Fig. 1 and Table 4). Usp2 was expressed approximately 3-fold more in wild-type *B. glumae* BGR1 cells than in cells of *tofI*::Ω mutant strain BGS2 after 24 h of incubation in Luria-Bertani (LB) medium (Table 4). When BGS2 was supplemented with 1 μM C_8-HSL, the expression of *usp2* recovered to the wild-type level (Fig. 1). This indicated that the expression of *usp2* is dependent on QS. Thus, we addressed questions regarding how *usp* genes are regulated and what the functional roles of Usps are in *B. glumae* BGR1.

II. Classification of Usps and their phylogenetic relationships

B. glumae strain BGR1 has 11 Usps classified as members of the Usp family in the Pfam database (Pfam accession number PF00582). The 11 Usps, which are encoded by the genes *usp1* to*11* (thus named to avoid confusion with the *uspA* and *uspC* to *uspG* genes of *E. coli*), are similar to those of *E. coli* and other *Burkholderia* species (Table 5).

To classify the Usps of *B. glumae* based on domain organization and

their characteristics, we compared them to those of *E. coli*. The Usps of *B. glumae* BGR1 can be divided into three classes based on domain organization (Fig. 2A). The first class consists of Usp1, Usp2, Usp4, Usp7, Usp9, and Usp10, which contain one conserved UspA domain and are similar in size to UspA of *E. coli* (Fig. 2A). Usp8 and Usp11 belong to the second class, which possesses one UspA domain and a conserved domain of unknown function at the C-terminus (Fig. 2A). The third class contains Usp3, Usp5, and Usp6, which possess two conserved UspA domains and are approximately twice the size of the UspA of *E. coli* (Fig. 2A and Table 5). The UspA domain in Usp2, Usp4, Usp8, Usp9, Usp10, and Usp11 possesses a conserved putative ATP-binding motif [G-2×-G-9×G(S/T)], whereas Usp1 and Usp7 have a similar motif, with some variation (Fig. 2B). In the third class of Usps, possessing two UspA domains, the conserved putative ATP-binding motif was not present in the first Usp domain but was found in the second domain, with some variation in Usp6 (Fig. 2B). Serine and threonine residues that are phosphorylation sites in *E. coli* seem to be conserved among Usps of *B. glumae*, except for Usp8 (Fig. 3).

We analyzed phylogenetic relationships using the amino acid sequences of the Usps of *B. glumae*. The Usps grouped into four separate clusters (Fig. 2C). Usp2 was distinct from the other Usp proteins and belonged to cluster I, and Usp10 and Usp11 belonged to cluster II. Usp1, Usp3, Usp5, Usp6, Usp7, and Usp9 belonged to cluster III, and Usp4 and Usp8 belonged to

Cluster IV (Fig. 2C). The level of identity among the Usps ranged from 14.3% to 48% (Table 6). When similarities among the Usps of *B. glumae* and *E. coli* were analyzed at the amino acid level, the Usps grouped into three clades (Fig. 2D). Usp3, Usp5, and Usp6 of *B. glumae* belonged to clade I, and all *E. coli* Usps and *B. glumae* Usp1 and Usp7 belonged to clade II. Usp2, Usp4, Usp8, Usp9, Usp10, and Usp11 of *B. glumae* formed clade III (Fig. 2D).

III. Genomic organization of the *usp* genes in *B. glumae*

The *usp1* gene was located in chromosome 1, and the other *usp* genes were located in chromosome 2. The *usp1*, *usp2*, and *usp7* genes were not clustered and were possibly expressed monocistronically (Fig. 4A to C), and the other *usp* genes were clustered in one region present in a cosmid, pBGQ12 (Fig. 4D). To determine whether any *usp* genes are cotranscribed with other genes, we performed reverse transcription (RT)-PCR using mRNA isolated from *B. glumae* strain BGR1 and the designated primers. The *usp3*, *usp4,* and *usp5* genes were co-transcribed with a histidine kinase gene (bglu_2g20220) and a two-component transcriptional regulator gene (bglu_2g20230) (Fig. 4D). The *usp8* gene was part of an operon with a hypothetical protein (bglu_2g20310) and a cyclic nucleotide-binding protein (bglu_2g20320) (Fig. 4D). The *usp9* gene was transcribed together with an acetoacetyl-coenzyme A (aceoacetyl-CoA) reductase (bglu_2g20290) (Fig. 4D).

IV. Phenotypes of *usp* mutants under stress conditions

To generate *usp* mutants and to determine how the expression of *usp* genes is regulated at the transcriptional level under different stress conditions, we generated Tn*3*-*gusA* gene fusions to each *usp* gene in wild-type strain BGR1, *tofI*::Ω mutant strain BGS2, and *qmsR*::Ω mutant strain BGS9 (Fig. 5 and Table 7). Using these mutants, we determined the functional roles of each Usp under different stress conditions, such as hydrogen peroxide, ternary butyl hydroperoxide (t-BOOH), cold and heat stress, mitomycin C, UV light, pH stress, osmotic shock with 1 M NaCl, and 4% ethanol. No increased sensitivity or resistance was detected in any of the *usp* mutants, except for heat shock, compared to the wild-type strain (Fig. 5 and Fig. 6). Under heat shock stress conditions, the populations of the *usp1* and *usp2* mutants rapidly decreased after 2 h and completely died after 3 h, whereas that of the wild-type strain BGR1 was maintained at approximately 1×10^{9} CFU ml^{-1} (Fig. 5). The populations of *usp7* and *usp8* mutants also decreased to 1×10^{7} CFU ml^{-1} and 2×10^{6} CFU ml^{-1}, respectively, but both mutants were far less sensitive to heat shock stress than the *usp1* and *usp2* mutants (Fig. 5). The survival rates of the other *usp* mutants were similar to that of the wild-type strain (Fig. 5). The death of the *usp1 usp2* double mutant cells under conditions of heat stress was more rapid than the death of cells of each single mutant (Fig. 5). Thus, *usp* genes may not be involved in oxidative stress defense or swarming and swimming motilities in *B.*

glumae (Fig. 6C and 6I and Fig. 7).

V. Expression of *usp* genes is regulated by QsmR

When the expression levels of each *usp* gene were measured with Tn*3*-*gusA* gene fusions to each *usp* gene in LB medium, the expression of all *usp* genes was 3- to 15-fold higher in wild-type strain BGR1 than in *tofI*::Ω mutant strain BGS2 and in *qmsR*::Ω mutant strain BGS9 (Table 7). The expression levels of *usp1* and *usp2* were higher than those of the other *usp* genes (Table 7). When 1 μM C_8-HSL was exogenously added to the cultures of each strain carrying the *usp*::Tn*3*-*gusA* fusion in the *tofI*::Ω mutant background, the expression levels were recovered to wild-type levels (Table 7). The lower level of expression of each of the *usp* genes in *qsmR*::Ω mutant strain BGS9 recovered to the level of the wild-type strain by providing pBGT63 carrying the *qsmR* gene in *trans* to each strain (Table 7). This indicated that the expression of *usp* genes is regulated by QS and, particularly, by QsmR.

To determine whether the expression of *usp* genes is regulated directly by QsmR, we performed electrophoretic mobility shift assays (EMSAs) with purified QsmR-His and the promoter regions of the *usp* genes. As shown in Fig. 8A and B and Fig. 9, QsmR-His specifically bound to the promoter regions of all *usp* genes, proving that the expression of the *usp* genes is activated directly by QsmR.

VI. Expression of *usp1* and *usp2* is dependent on RpoS

Because the *usp1* and *usp2* genes play important roles in the response to heat shock stress, we investigated the expression levels of these genes at different growth stages and under heat shock stress conditions in LB medium. The expression levels of both genes increased in a QsmR-dependent manner as cells entered the stationary phase (Fig. 10A and C). Because both genes were highly expressed in the stationary phase, we determined whether their expression were dependent on RpoS. The expression level of *usp1* was approximately 2-fold lower in strain BGO6 (*usp1*::Tn*3-gusA20, rpoS*::Gm^r) than in strain BGQ8 (*usp1*::Tn*3-gusA20*) (Fig. 10B), whereas that of *usp2* was approximately 5-fold lower in strain BGO7 (*usp2*::Tn*3-gusA104, rpoS*::Gm^r) than in strain BGQ9 (*usp2*::Tn*3-gusA104*) (Fig. 10D). This indicated that the expressions of both genes are dependent on RpoS.

To determine whether RpoS is involved directly in the expressions of the *usp1* and *usp2* genes, we performed EMSAs using the promoter regions of the genes. We observed DNA band shifts with 500 nM His-RpoS (complex I [CI]) and 50 nM or 100 nM *E. coli* RNA core enzyme (CII), which indicates that they bind to each 340-bp promoter region of the *usp1* and *usp2* genes (Fig. 8C and D). His-RpoS at 500 nM together with 50 nM *E. coli* RNA core polymerase bound to the same region and appeared at positions similar to those of samples treated with 50 nM or 100 nM *E. coli* RNA core polymerase alone (Fig. 8C and

D). However, shifted bands appeared in a higher position (CIII) when samples were treated with 500 nM His-RpoS and 100 nM *E. coli* RNA core enzyme, and heparin disturbed the direct binding of RpoS-RNA holoenzyme (Fig. 8C and D). This indicates that His-RpoS-RNA holoenzyme specifically binds to each 340-bp promoter region of the *usp1* and *usp2* genes.

To determine whether QS regulates *rpoS* expression, we measured *rpoS* expression levels in strains S70 (*rpoS*::Tn*3-gusA70*), S2S70 (*tofI*::Ω *rpoS*::Tn*3-gusA70*), and S9S70 (*qsmR*::Ω *rpoS*::Tn*3-gusA70*). The expression levels in strains S2S70 and S9S70 were no different than that in the wild-type strain (Fig. 11), indicating that *rpoS* expression is independent of QS.

VII. Expression of *usp1* is upregulated by a temperature shift

To determine whether the expressions of *usp1* and *usp2* are regulated by temperature stress, we measured the changes in the expression levels of the two genes after cold and heat shock stresses at 28℃ and 45℃, respectively. The expression level of the *usp1* gene was consistently about 2- or 3-fold higher under cold and heat shock conditions than under normal growth conditions at 37℃ (Fig. 12A). In addition, the upregulation of *usp1* by temperature stress was dependent on QsmR, whereas that of *usp2* was independent of temperature stress (Fig. 12A and B).

VIII. Mutants of *usp1* and *usp2* retain full virulence

To determine whether Usp1 and Usp2 play any role in the virulence of *B*. *glumae*, we inoculated the *usp1* or *usp2* mutant strains into rice panicles. Both mutants were highly virulent to the level of the wild-type (Fig. 13), indicating that neither protein affects the virulence of *B*. *glumae*.

DISCUSSION

The numbers of *usp* genes in Gram-negative bacteria are diverse, ranging from 1 to 12 (Boes *et al*., 2006; Chen *et al*., 2006; Hingley-Weilson *et al*., 2010; Mot *et al*., 2007; O`Toole *et al*., 2003), indicating that some functional redundancies of Usps exist or that each Usp might have a different role. We characterized the 11 *usp* genes identified in *B. glumae* BGR1. The genes exhibited relatively low levels of identity among each other; however, low levels of identity among known Usps at the amino acid level seem to be common. This indicates that predictions of Usp paralogs or orthologs based on similarities of amino acid sequences from various bacterial species may not be a preferred method of characterizing *usp* genes. In addition, comparisons of the Usps of *B. glumae* and *E. coli* provided no valuable information for predicting the possible functions of Usps. Thus, a more thorough characterization of these Usps is required for predicting which gene is a bona fide *usp* gene and for avoiding the misclassification of these genes, as happened with the '*uspB*' gene in *E. coli*, which was subsequently found not to be a bona fide *usp* gene (Gustavsson *et al*., 2002). Thus, we cannot rule out that some of the annotated *usp* genes in *B. glumae* may not be bona fide *usp* genes.

There are many different types of Usp family members in bacteria, archaea, and plants (Kvint *et al*., 2003). The Usps of bacteria are relatively

small and simple compared to those of archaea, cyanobacteria, and plants (O`Toole *et al*., 2003). The domain organization of the 11 Usps identified in *B*. *glumae* in the present study is similar to that of Usps of *E. coli* and *Mycobacterium tuberculosis* (O`Toole *et al*., 2003). Three Usps possess two Usp domains in tandem, but the other eight have one Usp domain. However, unlike the four Usps that have two Usp domains carrying two ATP-binding motifs in *M*. *tuberculosis*, only one ATP-binding motif was found for one of the two Usp domains of Usp3, Usp5, and Usp6 in *B*. *glumae*. The relationship between the organization of the Usp domain and the slight variation in the ATP-binding motif in the Usp domain and its effect on the biochemical functions of Usps remain unclear, because the biochemical properties of Usps are limited. From the limited information on the biochemical properties of Usps, posttranslational modification seems to be common (Freestone *et al*., 1997). The Phosphorylation of UspA and UspG was not unexpected because of the presence of the conserved ATP-binding motif. It has been known that serine or threonine of UspA is phosphorylated in response to stasis (Freestone *et al*., 1997). Interestingly, we identified one conserved serine or threonine residue from the alignment of amino acid residues of UspA and all 11 Usps in *B*. *glumae* (Fig. 3). This residue would be a strong candidate for a phosphorylation site of Usps.

Some aspects of the functional roles of Usps have been relatively well

characterized for *E. coli* compared to other organisms. However, information regarding the functions of Usps in other bacteria is very limited. The Usps of *E. coli* have individual roles, with some overlap, whereas those of *B. glumae* (except for Usp1 and Usp2) have no apparent major role under diverse stress conditions. In *E. coli*, the sensitivities of the *uspA* mutant and the wild-type during a temperature shift from 28℃ to 50℃ were indistinguishable (Nystrom *et al.*, 1994). In some cases, wild-type cells preadapted to a nonlethal temperature of 42℃ for 15 min were somewhat more resistant than preadapted *uspA* mutant cells (Nystrom *et al.*, 1994). However, the sensitivities of the *usp1* and *usp2* mutants to heat shock are clearly distinguishable in *B. glumae*. This indicates that Usp1 and Usp2 play major roles in protection against heat shock stress in *B. glumae*. The finding that the *usp7* and *usp8* mutant populations of *B. glumae* decreased approximately 100-fold 3 h after heat treatment indicates that Usp7 and Usp8 might be associated with the Usp1- and Usp2-mediated heat shock response or may play minor roles in the response to heat stress.

The biochemical mechanisms through which Usp1 and Usp2 confer resistance to heat shock stress in *B. glumae* are not clear. One plausible mechanism is that they might interact with GroEL to form proper protein conformations, thereby preventing unwanted protein aggregation under conditions of heat shock stress, similarly to how UspG in *E. coli* forms complexes with GroEL (Bochkareva *et al.*, 2002). However, this does not

explain the biochemical properties of Usp1 and Usp2.

The *uspA* genes in many bacteria are coordinately expressed by ppGpp, FadR, FtsK, RecA, CspC, and CspE under different conditions (Kvint *et al*., 2003). However, the regulation of *usp* genes in *B. glumae* is very different from that in *E. coli*. First, we showed that the expression of all *usp* genes in *B. glumae* is positively regulated by QS, specifically by QsmR. This is the first report of such QS regulation. Second, we demonstrated that the expression of *usp1* and *usp2* is dependent on RpoS, suggesting that RpoS plays a role in *usp* gene expression at entry into the stationary phase. In contrast, in *E. coli*, the expression of *uspA* is dependent on σ^{70} (Nystrom *et al*., 1994) and that of *uspB* during ethanol stress is dependent on σ^{s} (Farewell *et al*., 1998); we excluded *uspB* because it is not a bona fide *usp* gene (Gustavsson *et al*., 2002). Third, the expression of *usp1* was affected by a temperature shift in a QS-dependent manner in LB medium, which has not been reported for any *usp* gene of *E. coli*. Finally, unlike the regulation of *uspA* by ppGpp in *E. coli*, ppGpp may not influence *usp* gene expression in *B. glumae*. This is consistent with our observation that mutations in *usp* genes in *B. glumae* under starvation conditions do not show phenotypes similar to those observed for *E. coli*. These findings provide new data on *usp* gene regulation, which may help elucidate how bacteria coordinate gene expression to cope with stress conditions.

UspA of *Salmonella* spp. was reported previously to be important for

virulence in mice and survival in host cells (Liu *et al*., 2007). However, *usp1* and *usp2* may not play important roles in the virulence of *B. glumae*, considering that Usp1 and Usp2 appear to play a role in overcoming heat shock stress, but no other stresses.

In conclusion, Usps in *B. glumae* play very limited roles in the responses against various stresses, compared to the roles of Usps in *E. coli*. This suggests that *usp* gene regulation is diverse in different bacteria. If Usps possess no enzymatic functions and are regulatory proteins, Usps may interact with other proteins in bacteria, because Usps do not possess DNA-binding motifs. This suggests that Usp-mediated networks might be present and would be important for an understanding of how Usps function to overcome various stresses. It would be very interesting to determine whether QS is involved in *usp* gene regulation in other bacteria. To understand additional biological roles of Usps, the biochemical properties of Usps should be further investigated.

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Table 1. Strains and plasmids used in this study.

Bacterial strain or plasmid	Genotype or phenotype[a]	Source or reference
Strains		
Escherichia coli		
DH5α	F^- Φ80d*lac*ZΔ*M15*Δ(*lac*ZYA-*argF*)*U169endA1* *recA1hsd1hsdR17*($r_k^- m_k^+$)*deoRthi*-1*supE44*λ^-*gyrA96 relA1*	Gibco BRL
C2110	*polA*, Nal^r	Stachel *et al*. (1985)
HB101	F^- *mcrBmrrhsdS20*($r_B^- m_B^-$)*recA13leuB6ara-14* *proA2lacY1galK2xyl-5 mtl-1 rpsL20*(Sm^r) *supE44*λ^-	Gibco BRL
BL21(DE3)	F^- *ompT* $hsdS_B$($r_B^- m_B^-$)*gal dcm* (DE3)	Novagen
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)
BGQ8/BGSQ8	BGR1 *usp1*::Tn*3-gusA20*/BGS2 *usp1*::Tn*3-gusA20*	This study

Continnued on following page

Table 1 – *Continnued*

/BGH8	/BGS9 *usp1*::Tn*3-gusA20*	
BGQ9/BGSQ9	BGR1 *usp2*::Tn*3-gusA104*/BGS2 *usp2*::Tn*3-gusA104*	This study
/BGH9	/BGS9 *usp2*::Tn*3-gusA104*	
BGQ10/BGSQ10	BGR1 *usp3*::Tn*3-gusA40*/BGS2 *usp3*::Tn*3-gusA40*	This study
/BGH10	/BGS9 *usp3*::Tn*3-gusA40*	
BGQ14/BGSQ14	BGR1 *usp6*::Tn*3-gusA242*/BGS2 *usp6*::Tn*3-gusA242*	This study
/BGH14	/BGS9 *usp6*::Tn*3-gusA242*	
BGQ12/BGSQ12	BGR1 *usp7*::Tn*3-gusA52*/BGS2 *usp7*::Tn*3-gusA52*	This study
/BGH12	/BGS9 *usp7*::Tn*3-gusA52*	
BGQ16/BGSQ16	BGR1 *usp8*::Tn*3-gusA192*/BGS2 *usp8*::Tn*3-gusA192*	This study
/BGH16	/BGS9 *usp8*::Tn*3-gusA192*	
BGQ18/BGSQ18	BGR1 *usp9*::Tn*3-gusA136*/BGS2 *usp9*::Tn*3-gusA136*	This study
/BGH18	/BGS9 *usp9*::Tn*3-gusA136*	
BGQ21/BGSQ21	BGR1 *usp10*::Tn*3-gusA94*/BGS2 *usp10*::Tn*3-gusA94*	This study
/BGH21	/BGS9 *usp10*::Tn*3-gusA94*	

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Table 1 – *Continnued*

BGQ22/BGSQ22	BGR1 *usp11*::Tn*3-gusA181*/BGS2 *usp11*::Tn*3-gusA181*	This study
/BGH22	/BGS9 *usp11*::Tn*3-gusA181*	
BGO6/BGSO6	BGQ8 *rpoS*::Gm^r/BGSQ8 *rpoS*::Gm^r	This study
/BGH6	/BGH8 *rpoS*::Gm^r	
BGO7/BGSO7	BGQ9 *rpoS*::Gm^r/BGSQ9 *rpoS*::Gm^r	This study
/BGH7	/BGH9 *rpoS*::Gm^r	
BGQ82	BGQ9 *usp1*::Ω	This study
S70/S2S70	BGR1 *rpoS*::Tn*3-gusA70*/BGS2 *rpoS*::Tn*3-gusA70*	This study
/S9S70	/BGS9 *rpoS*::Tn*3-gusA70*	
Plasmids		
pRK2013	Tra^+; ColE1 replicon; Km^r	Figurski *et al*. (1979)
pHoKmGus	Promoterless β-glucuronidase gene; Km^r Amp^r *tnpA*	Bonas *et al*. (1989)
pSShe	Cm^r	Stachel *et al*. (1985)
pLysS	Encodes T7 lysozyme gene; Cm^r	Novagen
pBluescript II	Cloning vehicle, phagemid, pUC derivative; Amp^r	Stratagene

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Table 1 – *Continnued*

SK(+)		
pLAFR3	Tra^-, Mob^+ RK2 replicon; Tet^r	Staskawicz *et al*. (1987)
pRK415	Mob^+ *lacZ* Tet^r	Keen *et al*. (1988)
pBSGm	0.8-kb DNA fragment harboring gentamycin cassette cloned into pBluescript II SK(+); Amp^r Gm^r	Kim. J-G
pBGT63	2.2-kb DNA fragment harboring *qsmR* cloned into pLAFR3	Kim *et al*. (2007)
pQSMR-His	*qsmR* in pET21b; Amp^r	Kim *et al*. (2007)
pHP45Ω	Ω cassette; Sp^r Sm^r	Prentki *et al*. (1984)
pBGQ8	21.5-kb DNA fragment harboring *usp1* in pLAFR3	This study
pBGQ9	21.5-kb DNA fragment harboring *usp2* in pLAFR3	This study
pBGQ11	20.1-kb DNA fragment harboring *usp7* in pLAFR3	This study
pBGQ12	19.4-kb DNA fragment harboring *usp3* to *usp6* and *usp8* to *usp11* in pLAFR3	This study
pRPOS	22.8-kb DNA fragment harboring *rpoS* in pLAFR3	This study
pRPOS1	0.8-kb gentamycin cassette was inserted into AfeI site in *rpoS* in	This study

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Table 1 – *Continnued*

	pRK415	
pUSP1	2.0-kb cassette was inserted into EcoNI site in *usp1* in pRK415	This study
pHS1	1,046-bp PCR product harboring the *usp1* and its promoter region cloned into pRK415	This study
pHS2	1,649-bp EcoRI DNA fragment harboring *usp2* and its promoter region cloned into pRK415	This study
pRPOS-His	*rpo*S in pET14b; Amp^r	This study

[a] Where more than one strain is listed in a single row, the corresponding phenotypes are separated by slashes. Amp^r, ampicillin resistance; Cm^r, chloramphenicol resistance; Km^r, kanamycin resistance; Nal^r, nalidixic acid resistance; Rif^r, rifampicin resistance; Sm^r, streptomycin resistance; Sp^r, spectinomycin resistance; Tet^r, tetracycline resistance; Gm^r, gentamycin resistance.

Table 2. Primers used for RT–PCR.

Primer name[a]	Sequence (5′→3′)
PCR1-F	TCGTGCAAACCAGCGAATCG
PCR1-R	ATCGCTTCCCCAGGAGCCAA
PCR2- F	TTTCCTACAGGCAGGCGGGG
PCR2-R	AACTGCGCGCCGATCTGATC
PCR3-F	TGATCGCGTTCCTGTGCTCG
PCR3-R	TTGAACGCGAAGTCGGAACC
PCR4-F	GGACTCGCTTAGCCGGCCAC
PCR4-R	GCCGCCATGATGGTTCGATA
PCR5-F	TTTCCTACAGGCAGGCGGGG
PCR5-R	AACTGCGCGCCGATCTGATC
PCR6-F	GTCAGCACCAGGATGCGCAG
PCR6-R	TTTTCGATCACCGTCACGCT
PCR7-F	TAGGGGGTCATGGGGCGATC
PCR7-R	GTGCCGGTGCTGATGTCGCA
PCR8-F	ATGCCAGACGTACCTCGCAGG
PCR8-R	GGCTGAAGCGGCTGGCTCGT
PCR9-F	GAGCGCGGCCACTGGAAAAT
PCR9-R	ACGCTGCCGGTGCTGATATC
PCR1-C	GACCGTTTCCAGCTTCAGGC
PCR2-C	GTGGCCGGCTAAGCGAGTCC
PCR3-C	CGGGCGTGTCGTCATCGATT
PCR4-C	CGCGTGTCAGACGTCGAGCT

[a] F, forward primer: R, reverse primer.

Table 3. Primers used for gel shift assays.

Primer name[a]	Sequence (5′→3′)
usp1F	AGCAGCATCCGCAGCGTGGT
usp1R	GATCGCCTCCGTTGCGAGAT
usp2F	GCCGATCTCGTAGCGCATCA
usp2R	GAGGGCTCCCTGGTGCGGTG
usp3-4-5F	ATAGCTCATTGCGACGGCTC
usp3-4-5R	CAGCGATGTATACCGGCACG
usp6F	TGACGGCTCCTTGTCGATCG
usp6R	CATCTGCACCGCGCTGGGCT
usp7F	GGCGGCTTGGACTGAGGACG
usp7R	GGGCGATTATAGAAACGGCT
usp8 and usp9F	TTGAGACTCCTTAGGGATCG
usp8 and usp9R	TGGCATGCATGTCGACTCCT
usp10F	TCGCCTCGGTCGCCAATCTG
usp10R	TGCCCTCGCCCGTGTCCCAC
usp11F	TGGCTTGATCGTGATGGGAA
usp11R	CGCCATGATGGTTCGATACA

[a] F, forward primer: R, reverse primer.

Table 4. Identification of Usp2 under the control of QS in *B. glumae*.

Spot number	Protein description[a] [*B. glumae*]	Accession number/ Gene ID	Matching sequence[b]	Observed migration[c]		Theoretical migration		Fold change[d]		
				Mol wt (kDa)	pI	Mol wt (kDa)	pI	Mean	SD	P-value[e]
Q09	UspA domain protein2	YP_0029077/ bglu_2g00500	ADVSSLILEAAT EYGADLLV	15.2	5.171	16.5	5.060	3.53	0.96	0.001

[a] Accession numbers and locus IDs were obtained from the *B. glumae* BGR1 genome database. [b] In all sequences, L represents the ambiguous pairs (I/L). [c]The molecular weight and pI values were estimated from the data in Figure 1. [d] The mean and standard deviation (SD) of the fold change was calculated by comparing spot intensities of the wild-type sample (*B. glumae* BGR1) to the autoinducer synthesis mutant (*B. glumae* BGS2) using the quantitative image analysis (PDQuest 2-D Analysis Software V 8.0). [e]Student's *t*-test.

Table 5. Annotation of *B. glumae* genes encoding Usps.

Genes[a]	Gene I.D.	No. of amino acid residues	Mol wt (kDa)	BLASTP E-value	Homologs [organism] (GenBank Accession No.)
usp1	bglu_1g18770	167	17.9	3e-83	UspA domain protein [*Burkholderia gladioli* BSR3] (YP_004360494)
usp2	bglu_2g00550	154	16.5	5e-65	UspA domain protein [*Burkholderia gladioli* BSR3] (YP_004348108)
usp3	bglu_2g20190	279	30.5	2e-123	Universal stress protein family protein [*Burkholderia gladioli* BSR3] (YP_004360736)
usp4	bglu_2g20200	186	19.4	1e-50	UspA [*Burkholderia gladioli* BSR3] (YP_004360735)
usp5	bglu_2g20210	279	30.6	9e-109	UspA domain protein [*Burkholderia gladioli* BSR3] (YP_004360734)
usp6	bglu_2g20360	276	29.9	2e-111	UspA domain-containing protein [*Burkholderia gladioli* BSR3] (YP_004360710)
usp7	bglu_2g14410	144	15.5	5e-56	UspA family protein [*Burkholderia gladioli* BSR3] (YP_004349101)
usp8	bglu_2g20300	165	16.8	3e-57	Universal stress protein family [*Burkholderia gladioli* BSR3] (YP_004360723)
usp9	bglu_2g20280	170	17.4	1e-57	UspA domain protein [*Burkholderia gladioli* BSR3] (YP_004360725)
usp10	bglu_2g20370	151	16.0	1e-50	Universal stress protein UspA [*Burkholderia gladioli* BSR3] (YP_004360709)
usp11	bglu_2g20270	162	17.4	1e-58	UspA [*Burkholderia gladioli* BSR3] (YP_004360726)

[a]Unified nomenclature for *B. glumae usp* genes.

Table 6. Identity among Usps in *B. glumae.*

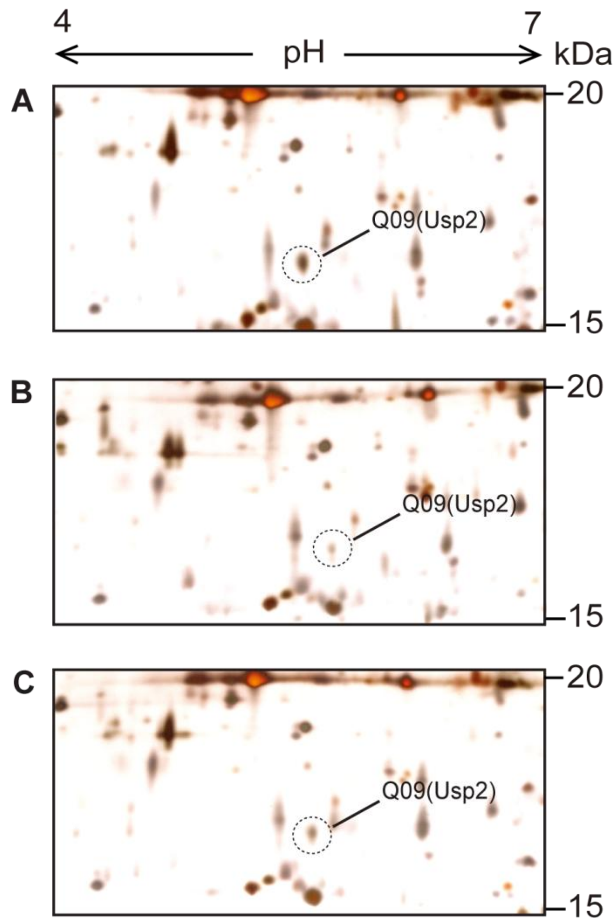
Proteins	Usp1	Usp2	Usp3	Usp4	Usp5	Usp6	Usp7	Usp8	Usp9	Usp10	Usp11
Usp1	-	18.1%	17.9%	21.8%	15.0%	16.5%	20.8%	23.3%	23.3%	23.3%	19.2%
Usp2	18.1%	-	18.1%	37.1%	15.1%	17.4%	20.9%	35.2%	29.4%	35.3%	33.3%
Usp3	17.9%	18.1%	-	19.1%	40.4%	42.3%	17.9%	20.8%	16.1%	17.6%	19.4%
Usp4	21.8%	37.1%	19.1%	-	20.0%	21.7%	15.6%	42.5%	27.2%	34.0%	30.1%
Usp5	15.0%	15.1%	40.4%	20.0%	-	48.0%	14.3%	17.6%	17.9%	18.0%	15.4%
Usp6	16.5%	17.4%	42.3%	21.7%	48%	-	17.8%	16.8%	17.4%	17.4%	19.6%
Usp7	20.8%	20.9%	17.9%	15.6%	14.3%	17.8%	-	18.5%	17.6%	23.7%	21.2%
Usp8	23.3%	35.2%	20.8%	42.5%	17.6%	16.8%	18.5%	-	31.4%	30.5%	35.8%
Usp9	23.3%	29.4%	16.1%	27.2%	17.9%	17.4%	17.6%	31.4%	-	31.0%	29.7%
Usp10	23.3%	35.3%	17.6%	34.0%	18.0%	17.4%	23.7%	30.5%	31.0%	-	37.8%
Usp11	19.2%	33.3%	19.4%	30.1%	15.4%	19.6%	21.2%	35.8%	29.7%	37.8%	-

Table 7. Expression of *usp* genes in *B. glumae*

Tn*3*-*gusA* fusions	Mean sp act of β-glucuronidase (10^{-11}U $CFU^{-1}min^{-1}$)±SD[a]				
	BGR1 (wild-type)	BGS2 (*tofI*::Ω)	BGS9 (*qsmR*::Ω)	BGS2 + 1 μM C_8-HSL	BGS9 (pBGT63)
usp1::Tn*3-gusA20*	137.6±10.0	34.6±7.2	30.7±4.5	112.3±5.6	76.5±9.2
usp2::Tn*3-gusA104*	364.8±9.8	24.0±3.3	55.5±7.8	185.4±6.4	152.0±8.7
usp3::Tn*3-gusA40*	48.4±3.0	16.1±0.4	17.3±0.6	50.8±5.4	40.0±2.8
usp6::Tn*3-gusA242*	35.1±1.1	9.18±0.2	12.4±0.8	39.6±2.3	25.8±1.7
usp7::Tn*3-gusA52*	35.2±0.9	7.0±0.1	9.59±0.9	34.8±1.4	40.4±1.9
usp8::Tn*3-gusA192*	61.9±5.8	19.5±0.7	12.7±0.8	81.7±3.1	48.0±2.9
usp9::Tn*3-gusA136*	61.1±0.9	22.3±1.1	30.0±1.0	65.1±4.9	50.3±3.7
usp10::Tn*3-gusA94*	54.4±7.2	17.1±0.5	9.65±0.6	65.0±3.7	49.2±4.2
usp11::Tn*3-gusA181*	52.1±4.1	16.8±0.8	12.7±0.8	65.5±5.1	52.3±4.3

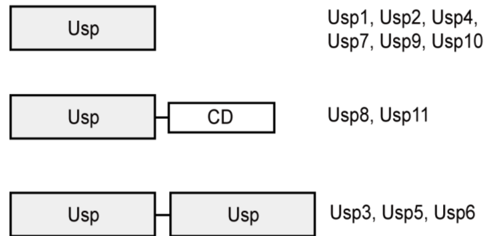
[a] Bacterial cells were grown for 12 h at 37℃. One unit of β-glucuronidase was defined as 1 nmol of 4-methyllumbelliferone released per bacterium per min. All values are means ± standard deviation (SD) of values from triplicate experiments.

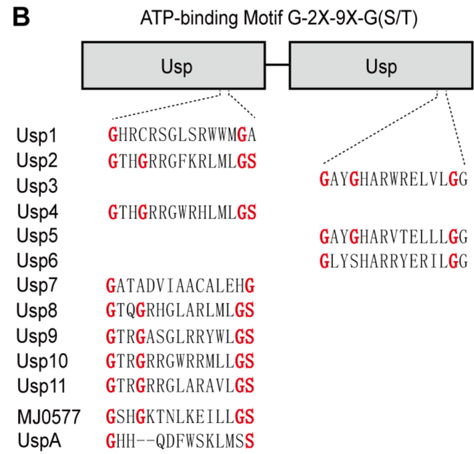
Fig. 1. Two-dimensional gel analysis of *B. glumae* cell extracts. (A) BGR1 (wild type). (B) BGS2 (*tofI*::Ω, *N*-octanoyl homoserine lactone [C_8-HSL] synthase mutant). (C) BGS2 cells grown in LB medium supplemented with 1 μM C_8-HSL. The cultures were harvested in the stationary phase. A total of 100 μg protein from bacterial cells was separated and stained with silver nitrate. Gels shown are representative of three independent experiments. Spot number Q09 was identified as Usp2.

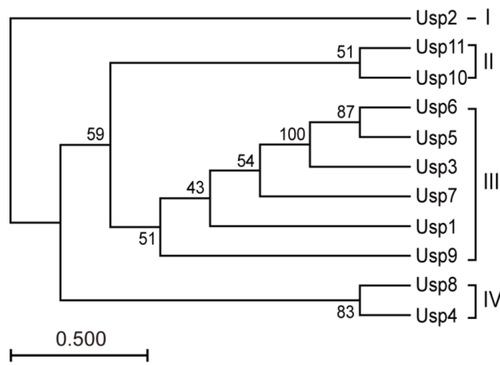


4
pH
7
kDa
A
20
Q09(Usp2)
15
B
20
Q09(Usp2)
15
C
20
Q09(Usp2)
15

Fig. 2. Phylogenetic relationships among Usps of *E. coli* and *B. glumae* and conserved ATP-binding motifs. (A) Classification of 11 Usps of *B. glumae* (Pfam accession number PF00582) based on domain organization. CD denotes a conserved domain of unknown function. (B) Conservation of the putative ATP-binding motif of MJ0577 (*Methanocaldococcus jannaschii* DSM 2661) Usp in the *B. glumae* Usps. The corresponding region of UspA in *E. coli* is shown for comparison. Conserved amino acid residues are in red. (C) Phylogenetic relationships among Usps of *B. glumae* using amino acid sequences. (D) Similarities of Usps in *B. glumae* and *E. coli* at the amino acid level. The phylogenetic tree calculation was performed by using the CLC main workbench 5.6 program, based on a sequence distance method that utilizes the neighbor-joining algorithm. Values at branches indicate the percent bootstrap support for 100 replicates.


A
Usp
Usp1, Usp2, Usp4, Usp7, Usp9, Usp10
Usp
CD
Usp8, Usp11
Usp
Usp
Usp3, Usp5, Usp6


B
ATP-binding Motif G-2X-9X-G(S/T)
Usp
Usp
Usp1 GHRCRSGLSRWWMGA
Usp2 GTHGRRGFKRLMLGS
Usp3 GAYGHARWRELVLGG
Usp4 GTHGRRGWRHLMLGS
Usp5 GAYGHARVTELLLGG
Usp6 GLYSHARRYERILGG
Usp7 GATADVIAACALEHG
Usp8 GTQGRHGLARLMLGS
Usp9 GTRGASGLRRYWLGS
Usp10 GTRGRRGWRRMLLGS
Usp11 GTRGRRGLARAVLGS
MJ0577 GSHGKTNLKEILLGS
UspA GHH--QDFWSKLMSS


C
Usp2 I
Usp11
Usp10
II
Usp6
Usp5
Usp3
Usp7
Usp1
Usp9
III
Usp8
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IV
51
59
87
100
54
43
51
83
0.500

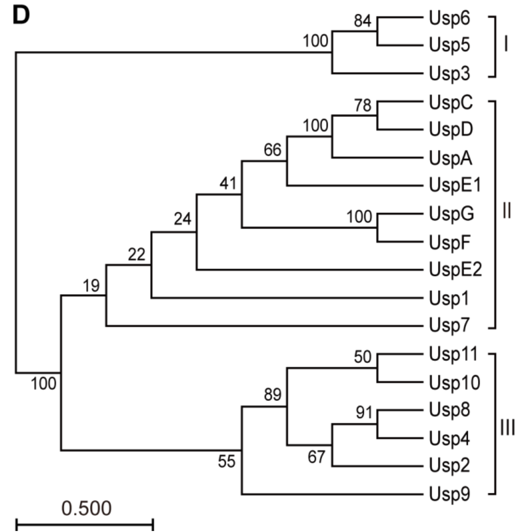
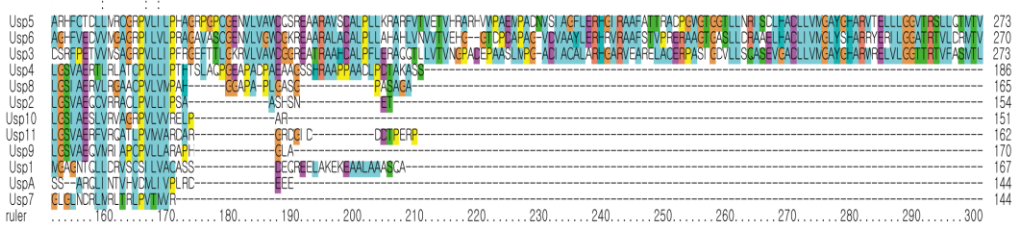
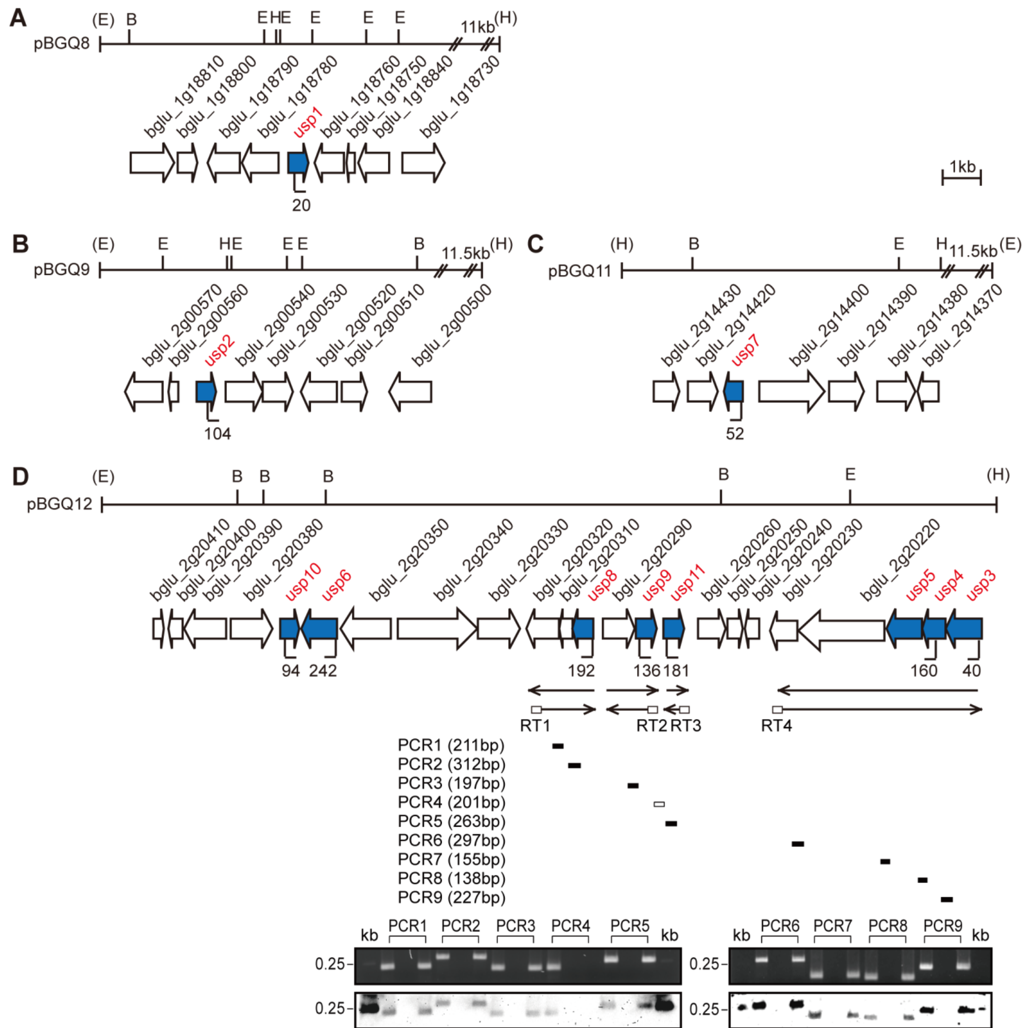

D
Usp6
Usp5
Usp3
I
UspC
UspD
UspA
UspE1
UspG
UspF
UspE2
Usp1
Usp7
II
Usp11
Usp10
Usp8
Usp4
Usp2
Usp9
III
84
100
78
100
66
41
100
24
22
19
50
89
91
67
55
100
0.500

Fig. 3. Prediction of serine and threonine residues in *B. glumae* Usps. Comparison of serine and threonine residues that are known phosphorylation sites in UspA of *E. coli* and those in *B. glumae*. Red arrow indicates a predicted phosphorylation site.



Usp5	PVLMSH	279
Usp6	PVLFSH	276
Usp3	PVLISH	279
Usp4	------	186
Usp8	------	165
Usp2	------	154
Usp10	------	151
Usp11	------	162
Usp9	------	170
Usp1	------	167
UspA	------	144
Usp7	------	144
ruler	......	

Fig. 4. Genetic organization of *usp* genes in *B. glumae.* (A to C) Schematic organization of *usp1*, *usp2*, and *usp7* genes. (D) Schematic organization of *usp3*, *usp4*, *usp5*, *usp6*, *usp8*, *usp9*, *usp10,* and *usp11* genes. Arrows below transcript arrows represent the direction and extent of cDNA. The short thick bars below the reverse transcription (RT) arrows indicate the nine polymerase chain reaction (PCR) products from the corresponding RT reactions. The expected sizes of the PCR products are indicated in parentheses for each PCR. Shown are data for agarose gel analysis (top) and Southern hybridization analysis (bottom) of the RT–PCR products. Southern hybridization was performed by using pBGQ12 as probe DNA. The first lane used chromosomal DNA, the second lane used total RNA, and the third lane used cDNA as a template for each PCR. Vertical bars in the map denote the positions and orientations of the Tn*3-gusA* insertions. The restriction enzyme sites are indicated as follows: E, EcoRI; B, BamHI; H, HindIII.



A
pBGQ8
(E) B E H E E E E 11kb (H)
bglu_1g18810
bglu_1g18800
bglu_1g18790
bglu_1g18780
usp1
bglu_1g18760
bglu_1g18750
bglu_1g18840
bglu_1g18730
20
1kb
B
pBGQ9
(E) E H E E E B 11.5kb (H)
bglu_2g00570
bglu_2g00560
usp2
bglu_2g00540
bglu_2g00530
bglu_2g00520
bglu_2g00510
bglu_2g00500
104
C
pBGQ11
(H) B E H 11.5kb (E)
bglu_2g14430
bglu_2g14420
usp7
bglu_2g14400
bglu_2g14390
bglu_2g14380
bglu_2g14370
52
D
pBGQ12
(E) B B B B E (H)
bglu_2g20410
bglu_2g20400
bglu_2g20390
bglu_2g20380
usp10
usp6
bglu_2g20350
bglu_2g20340
bglu_2g20330
bglu_2g20320
bglu_2g20310
usp8
bglu_2g20290
usp9
usp11
bglu_2g20260
bglu_2g20250
bglu_2g20240
bglu_2g20230
bglu_2g20220
usp5
usp4
usp3
94 242 192 136 181 160 40
RT1 RT2 RT3 RT4
PCR1 (211bp)
PCR2 (312bp)
PCR3 (197bp)
PCR4 (201bp)
PCR5 (263bp)
PCR6 (297bp)
PCR7 (155bp)
PCR8 (138bp)
PCR9 (227bp)
kb PCR1 PCR2 PCR3 PCR4 PCR5 kb
kb PCR6 PCR7 PCR8 PCR9 kb
0.25

Fig. 5. Survival of *usp* mutants after exposure to heat shock in the stationary phase. All *B*. *glumae* BGR1 derivatives were cultured at 37℃, and then shifted to 45℃ to expose to heat shock stress. All experiments were performed by using three independent cultures; error bars represent the standard deviations.

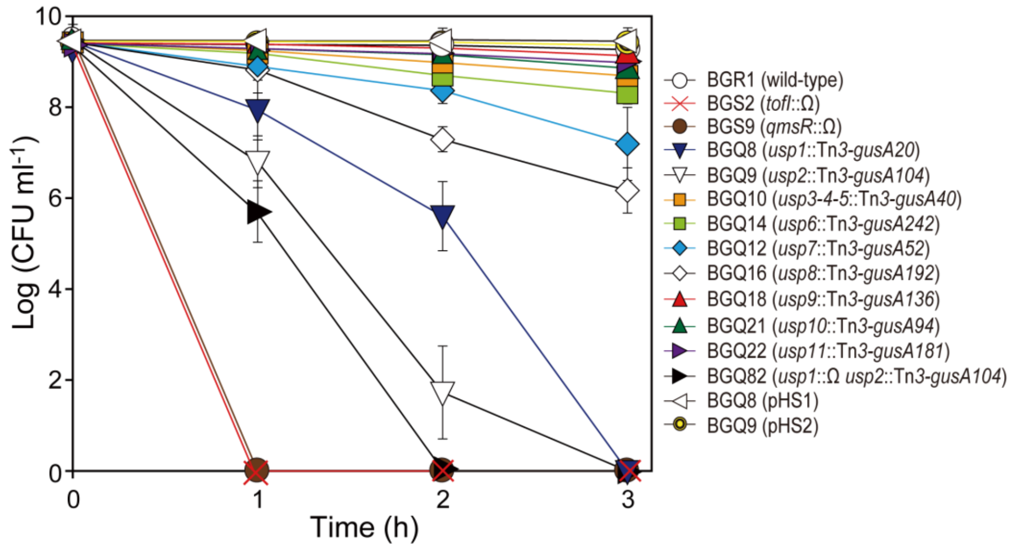

10
8
6
4
2
0
Log (CFU ml-1)
0
1
2
3
Time (h)
BGR1 (wild-type)
BGS2 (tofI::Ω)
BGS9 (qmsR::Ω)
BGQ8 (usp1::Tn3-gusA20)
BGQ9 (usp2::Tn3-gusA104)
BGQ10 (usp3-4-5::Tn3-gusA40)
BGQ14 (usp6::Tn3-gusA242)
BGQ12 (usp7::Tn3-gusA52)
BGQ16 (usp8::Tn3-gusA192)
BGQ18 (usp9::Tn3-gusA136)
BGQ21 (usp10::Tn3-gusA94)
BGQ22 (usp11::Tn3-gusA181)
BGQ82 (usp1::Ω usp2::Tn3-gusA104)
BGQ8 (pHS1)
BGQ9 (pHS2)

Fig. 6. Comparison of the stress sensitivity of *usp1* and *usp2* mutants. Cells were grown in LB medium and allowed to reach the stationary phase before growth stress was initiated. (A) 1 M NaCl, (B) 4% ethanol (EtOH), (C) H_2O_2, (D) cold shock, (E) long-term survival, (F) mitomycin C, (G) pH 4 shock, (H) pH 10 shock, (I) t-BOOH, (J) ultraviolet (UV) irradiation.

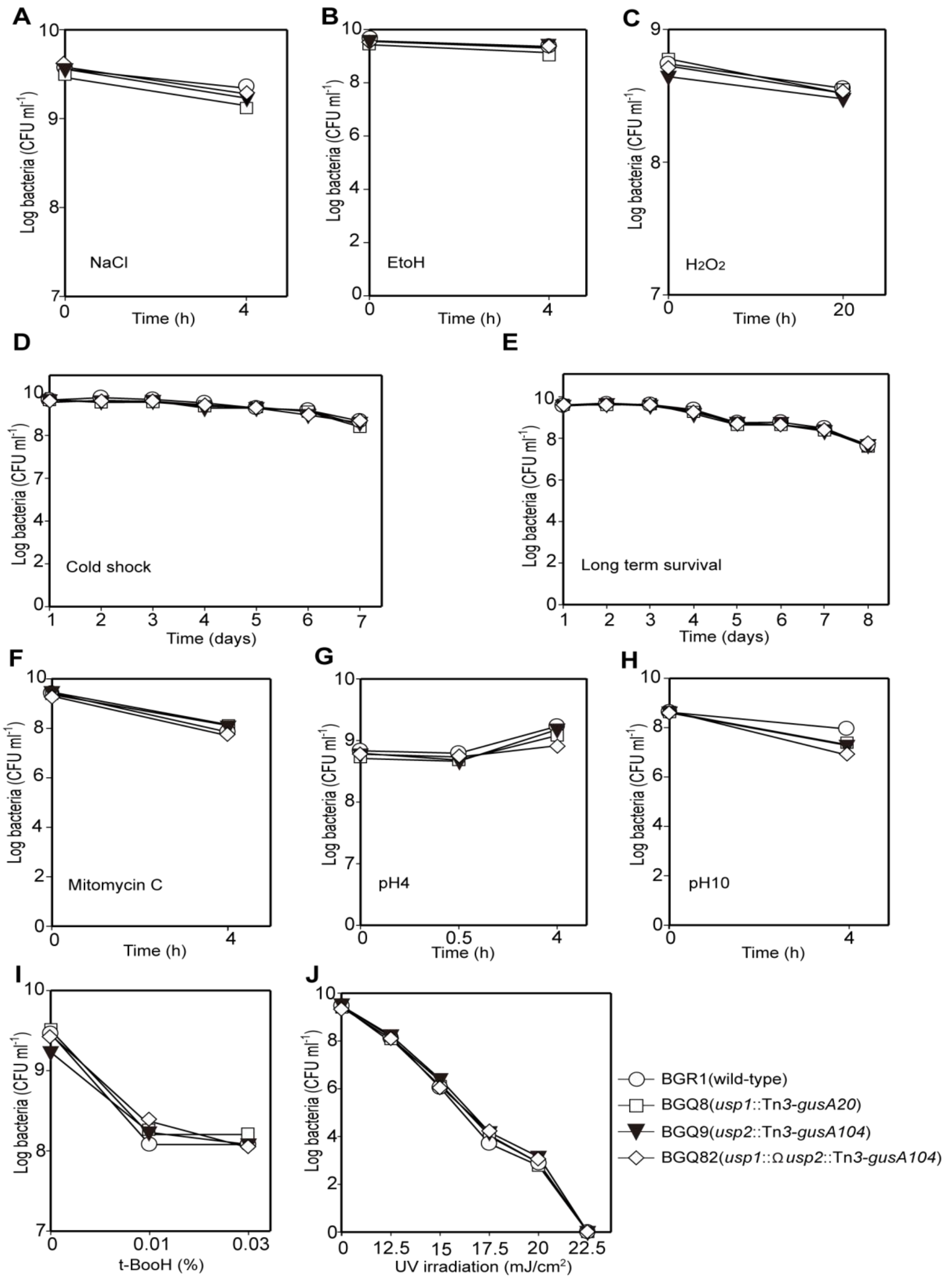

A
NaCl
Time (h)
Log bacteria (CFU ml-1)
B
EtoH
C
H2O2
D
Cold shock
Time (days)
E
Long term survival
F
Mitomycin C
G
pH4
H
pH10
I
t-BooH (%)
J
UV irradiation (mJ/cm2)
BGR1(wild-type)
BGQ8(usp1::Tn3-gusA20)
BGQ9(usp2::Tn3-gusA104)
BGQ82(usp1::Ωusp2::Tn3-gusA104)

Fig. 7. Swimming and swarming motilities of *usp* mutants in *B. glumae*. Swimming motility and swarming motility are observed in BGR1 wild-type and all *usp* mutants, except for quorum sensing-deficient mutants in BGS2 and BGS9 at 37℃ for 12 h.

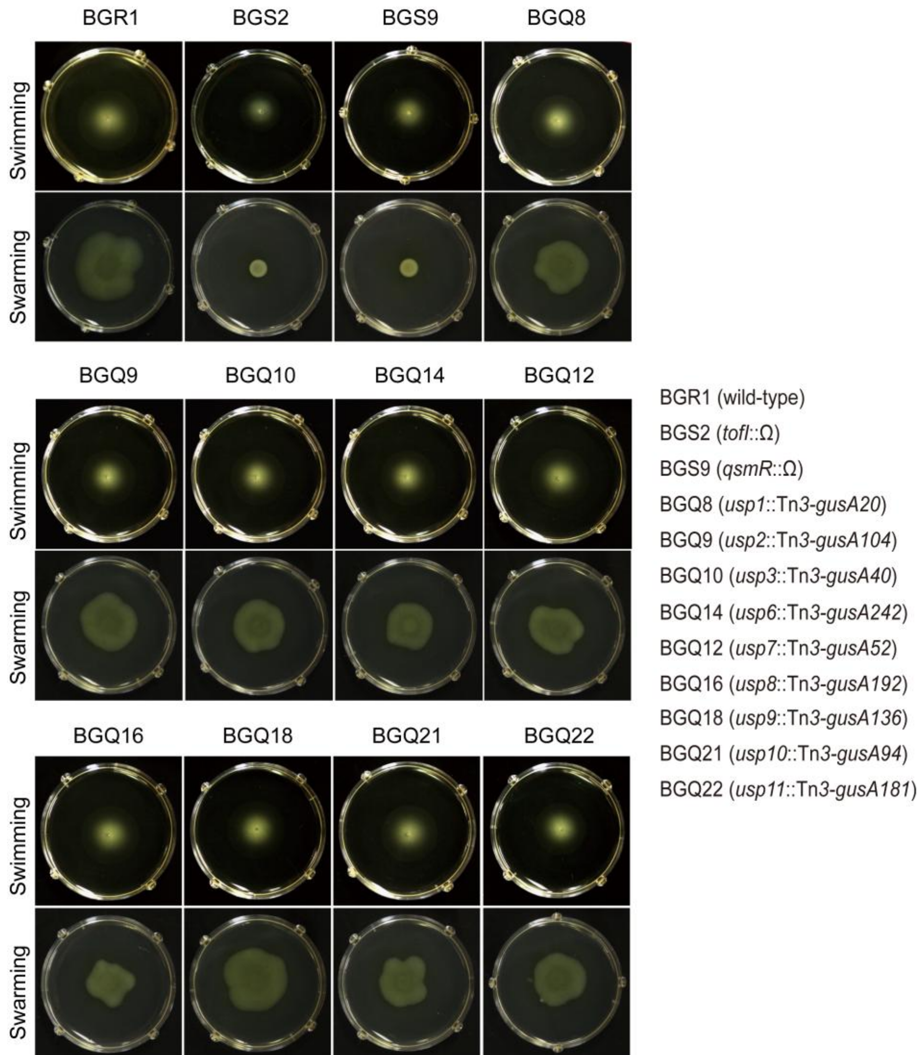
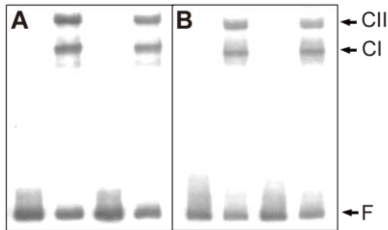

BGR1
BGS2
BGS9
BGQ8
Swimming
Swarming
BGQ9
BGQ10
BGQ14
BGQ12
Swimming
Swarming
BGQ16
BGQ18
BGQ21
BGQ22
Swimming
Swarming
BGR1 (wild-type)
BGS2 (*tofI*::Ω)
BGS9 (*qsmR*::Ω)
BGQ8 (*usp1*::Tn*3-gusA20*)
BGQ9 (*usp2*::Tn*3-gusA104*)
BGQ10 (*usp3*::Tn*3-gusA40*)
BGQ14 (*usp6*::Tn*3-gusA242*)
BGQ12 (*usp7*::Tn*3-gusA52*)
BGQ16 (*usp8*::Tn*3-gusA192*)
BGQ18 (*usp9*::Tn*3-gusA136*)
BGQ21 (*usp10*::Tn*3-gusA94*)
BGQ22 (*usp11*::Tn*3-gusA181*)

Fig. 8. Gel mobility shift assay of the *usp1* and *usp2* regulatory regions using QsmR-His, His-RpoS, and *E. coli* RNA core enzyme. (A and B) Binding of QsmR-His to the *usp1* (A) and *usp2* (B) regulatory regions. (C and D) Binding of RpoS, core enzyme, or RpoS-RNA holoenzyme to the *usp1* (C) and *usp2* (D) regulatory regions. CI, CII, and CIII indicate DNA-protein complex I, II, and III, respectively, and F denotes free DNA.


QsmR-His (250 nM) − + + + − + + +
Labeled target promoter region (1 nM) + + + + + + + +
Unlabeld target promoter region (27 nM) − − + − − − + −
Unlabeled *katE* promoter region (1 nM) − − − + − − − +
A
B
CII
CI
F

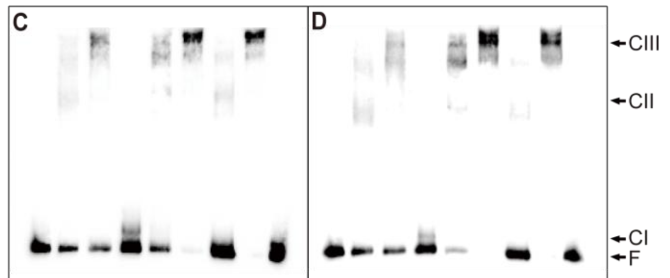

His-RpoS (500 nM) - - - + + + + + + - - - + + + + + +
Core enzyme (nM) - + + - + + + + + - + + - + + + + +
(50)(100) (50) (100) (50)(100) (50) (100)
Heparin (0.05 mg ml-1) - - - - - - - - + - - - - - - - - +
Unlabeled target promoter region (23 nM) - - - - - - + - - - - - - - - + - -
Unlabeled *katE* promoter region (1 nM) - - - - - - - + - - - - - - - - + -
Labeled target promoter region (1 nM) + + + + + + + + + + + + + + + + + +
C
D
CIII
CII
CI
F

Fig. 9. Gel mobility shift assay using purified QsmR-His and a DNA fragment that contained the *B. glumae usp3* to *usp5, usp6, usp7, usp8* and *usp9, usp10,* and *usp11* regulatory regions. (A) *usp3* to *usp5* regulatory region, (B) *usp6* regulatory region, (C) *usp7* regulatory region, (D) *usp8* and *usp9* regulatory region, (E) *usp10* regulatory region, (F) *usp11* regulatory region. Lane 1, no protein and labeled *usp3* to *usp11* regulatory regions; Lane 2, QsmR-His (250 nM) plus labeled *usp3* to *usp11* regulatory regions; Lane 3, QsmR-His (250 nM) plus labeled *usp3* to *usp11* regulatory regions plus unlabeled *usp1* regulatory region; Lane 4, QsmR-His (250 nM) plus labeled *usp3* to *usp11* regulatory regions plus unlabeled *katE* regulatory region. C and F denote the DNA-protein complex and free DNA, respectively. CI and CII indicate DNA-protein complex I and II, respectively.

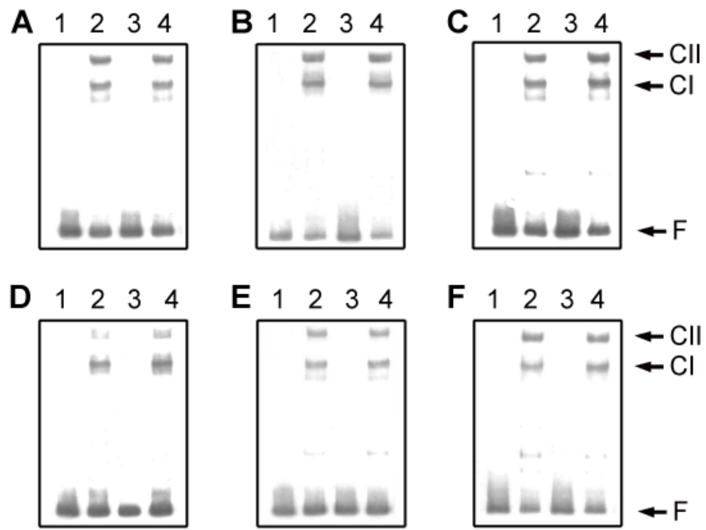
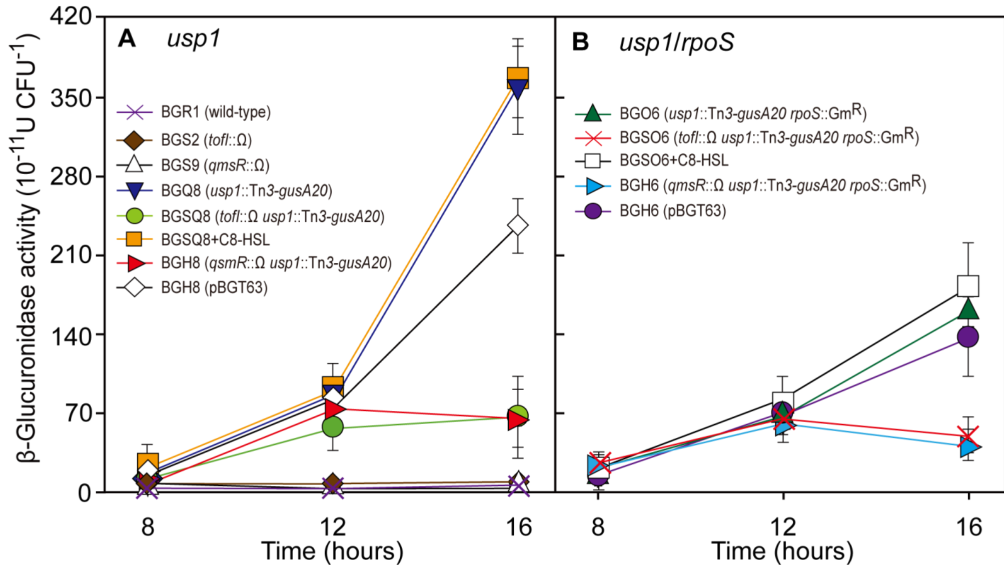

A
1
2
3
4
B
C
CII
CI
F
D
E
F

Fig. 10. Expression of the *usp1* and *usp2* genes at different growth phases and in different genetic backgrounds. (A and B) The expression level of *usp1* was elevated in the stationary phase and was dependent on QsmR and RpoS. (C and D) The *usp2* gene was highly expressed in the stationary phase and in a QsmR- and RpoS-dependent manner.


A usp1
B usp1/rpoS
β-Glucuronidase activity (10-11U CFU-1)
BGR1 (wild-type)
BGS2 (tofI::Ω)
BGS9 (qmsR::Ω)
BGQ8 (usp1::Tn3-gusA20)
BGSQ8 (tofI::Ω usp1::Tn3-gusA20)
BGSQ8+C8-HSL
BGH8 (qsmR::Ω usp1::Tn3-gusA20)
BGH8 (pBGT63)
BGO6 (usp1::Tn3-gusA20 rpoS::GmR)
BGSO6 (tofI::Ω usp1::Tn3-gusA20 rpoS::GmR)
BGSO6+C8-HSL
BGH6 (qmsR::Ω usp1::Tn3-gusA20 rpoS::GmR)
BGH6 (pBGT63)
Time (hours)

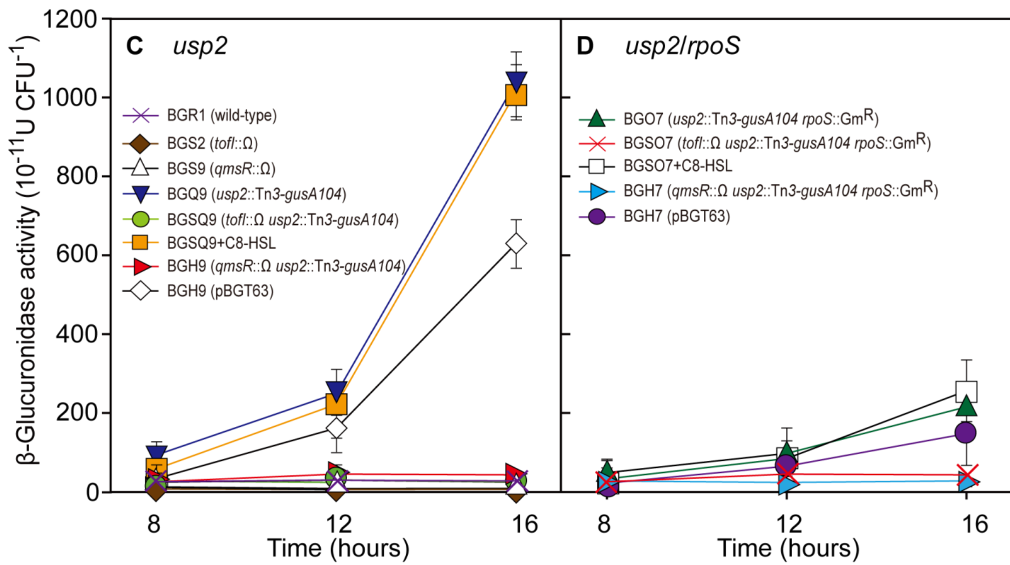

C usp2
D usp2/rpoS
β-Glucuronidase activity (10-11U CFU-1)
BGR1 (wild-type)
BGS2 (tofI::Ω)
BGS9 (qmsR::Ω)
BGQ9 (usp2::Tn3-gusA104)
BGSQ9 (tofI::Ω usp2::Tn3-gusA104)
BGSQ9+C8-HSL
BGH9 (qmsR::Ω usp2::Tn3-gusA104)
BGH9 (pBGT63)
BGO7 (usp2::Tn3-gusA104 rpoS::GmR)
BGSO7 (tofI::Ω usp2::Tn3-gusA104 rpoS::GmR)
BGSO7+C8-HSL
BGH7 (qmsR::Ω usp2::Tn3-gusA104 rpoS::GmR)
BGH7 (pBGT63)
Time (hours)

Fig. 11. Expression of *rpoS* is independent of QS. Cells were grown in LB medium and allowed to reach the stationary phase.

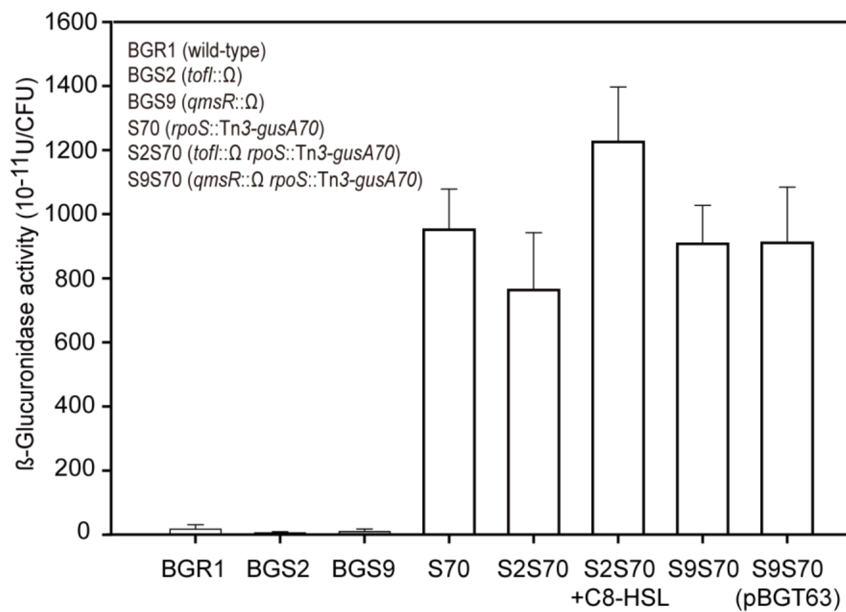
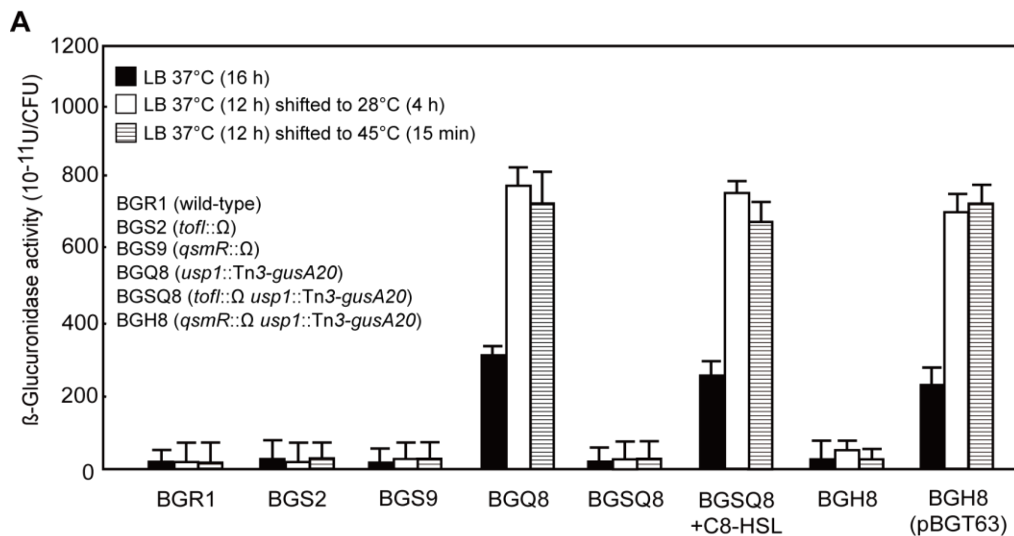

1600
1400
1200
1000
800
600
400
200
0
ß-Glucuronidase activity (10-11U/CFU)
BGR1 (wild-type)
BGS2 (tofI::Ω)
BGS9 (qmsR::Ω)
S70 (rpoS::Tn3-gusA70)
S2S70 (tofI::Ω rpoS::Tn3-gusA70)
S9S70 (qmsR::Ω rpoS::Tn3-gusA70)
BGR1
BGS2
BGS9
S70
S2S70
S2S70 +C8-HSL
S9S70
S9S70 (pBGT63)

Fig. 12. Expression of the *usp1* and *usp2* genes under conditions of a temperature shift. (A) Expression of *usp1* was activated by QsmR and upregulated by cold and heat shock stresses. (B) Expression of *usp2* was dependent on QsmR, and the expression pattern was not influenced by a temperature shift.


A
β-Glucuronidase activity (10-11U/CFU)
LB 37°C (16 h)
LB 37°C (12 h) shifted to 28°C (4 h)
LB 37°C (12 h) shifted to 45°C (15 min)
BGR1 (wild-type)
BGS2 (tofI::Ω)
BGS9 (qsmR::Ω)
BGQ8 (usp1::Tn3-gusA20)
BGSQ8 (tofI::Ω usp1::Tn3-gusA20)
BGH8 (qsmR::Ω usp1::Tn3-gusA20)
0
200
400
600
800
1000
1200
BGR1
BGS2
BGS9
BGQ8
BGSQ8
BGSQ8 +C8-HSL
BGH8
BGH8 (pBGT63)

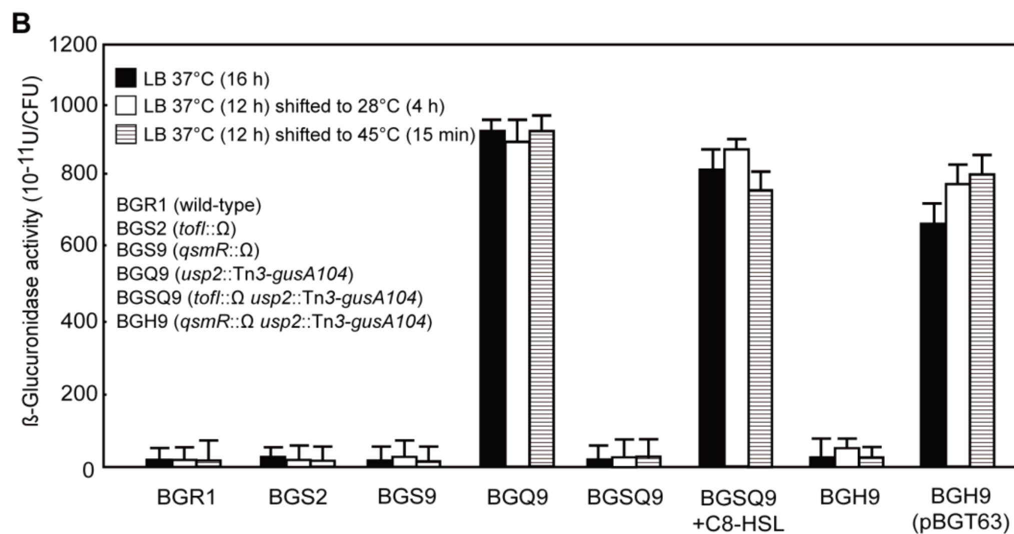
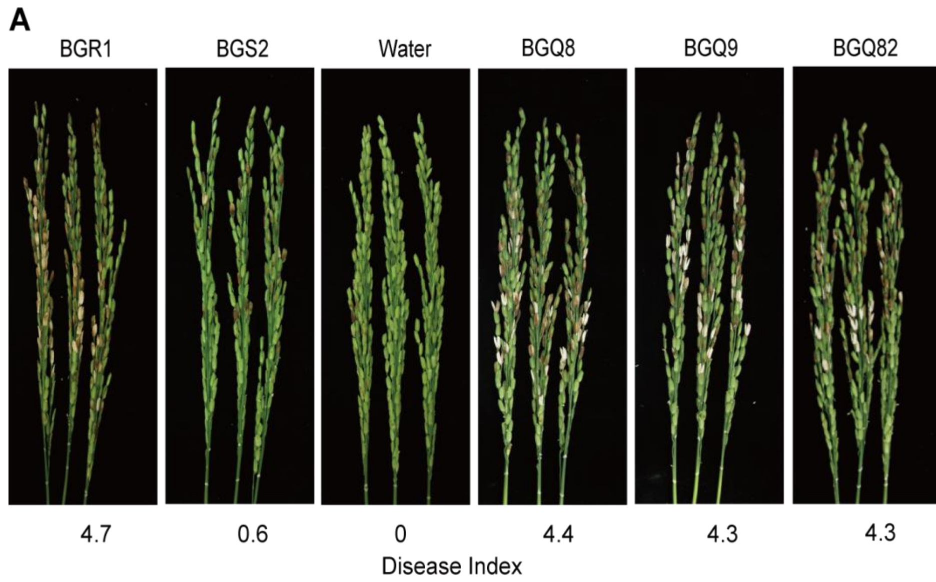
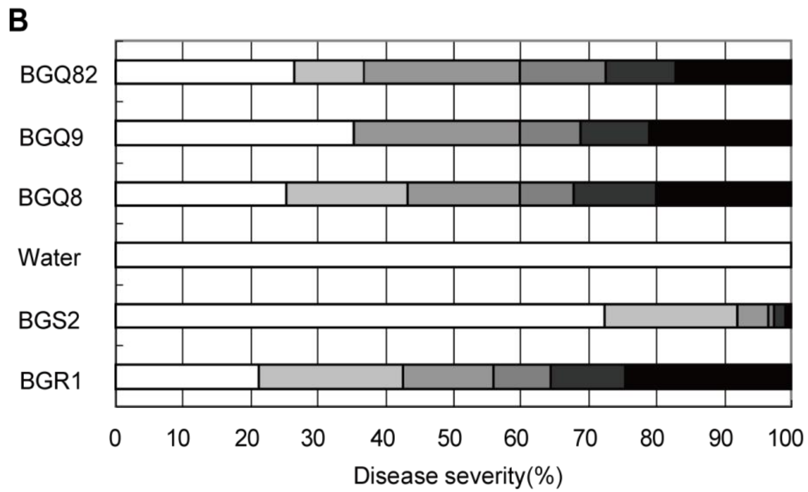

B
β-Glucuronidase activity (10-11U/CFU)
LB 37°C (16 h)
LB 37°C (12 h) shifted to 28°C (4 h)
LB 37°C (12 h) shifted to 45°C (15 min)
BGR1 (wild-type)
BGS2 (tofI::Ω)
BGS9 (qsmR::Ω)
BGQ9 (usp2::Tn3-gusA104)
BGSQ9 (tofI::Ω usp2::Tn3-gusA104)
BGH9 (qsmR::Ω usp2::Tn3-gusA104)
0
200
400
600
800
1000
1200
BGR1
BGS2
BGS9
BGQ9
BGSQ9
BGSQ9 +C8-HSL
BGH9
BGH9 (pBGT63)

Fig. 13. Pathogenicity of the *usp1* and *usp2* mutants. Panicles of rice plant (*O. sativa* cv. Milyang23) were inoculated with *B. glumae* strain BGR1 (wild-type), BGS2 (*tofI*::Ω), BGQ8 (*usp1*::Tn*3-gusA20*), BGQ9 (*usp2*::Tn*3-gusA104*), or BGQ82 (*usp1*::Ω *usp2*::Tn*3-gusA104*). (B) Distribution patterns of disease severity for each treatment. The photograph was taken 7 days after inoculation. The pathogenicity assay was repeated three times with replications. The disease index of the tested rice plants is described in the Materials and Methods.


A
BGR1
BGS2
Water
BGQ8
BGQ9
BGQ82
4.7
0.6
0
4.4
4.3
4.3
Disease Index


B
BGQ82
BGQ9
BGQ8
Water
BGS2
BGR1
0
1
2
3
4
5
0 10 20 30 40 50 60 70 80 90 100
Disease severity(%)

*Burkholderia glumae*의 Quorum Sensing과 RpoS에 의한 Universal Stress Protein 유전자의 발현조절

김홍섭

초 록

대부분의 세균들은 살아가면서 여러가지 환경적인 스트레스 즉, 영양결핍, 온도 스트레스, 산화적 스트레스 그리고 독성 물질등과 같은 스트레스에 노출되어 있다. 이런 환경적인 스트레스를 극복하기 위해서 세균들은 스스로를 방어하기 위한 기작을 가지고 있다. 위와 같은 여러가지 스트레스에 반응하는 유전자 중 하나가 universal stress protein 인 *usp* 유전자이다.

본 연구는 세균성 벼알마름병을 일으키는 *Burkholderia glumae*가 quorum sensing (QS)이라는 밀도인식 기작과 stationary phase sigma factor로 알려져 있는 Rpos에 의해서 universal stress protein 유전자의 발현조절 기작과 여러가지 스트레스 중 heat shock

stress에 중요한 역할을 한다는 사실을 밝혔다.

*B. glumae*은 신호물질로 N-octanoyl-homoserine lactone (C_8-HSL)을 사용하여 cell과 cell 사이에 의사소통을 하는 TofI/TofR QS system을 가지고 있다. *tofI*는 autoinducer인 C_8-HSL를 합성하는 유전자이며, *tofR*은 이 autoinducer의 receptor 유전자이다. 이 TofR/C_8-HSL의 복합체는 toxoflavin의 생합성과 IclR type transcriptional regulator인 *qsmR*을 조절한다. 우리는 *B. glumae*의 QS에 의존적인 단백질체 분석을 통하여 universal stress protein들 중 Usp2 단백질을 동정하였다. *B. glumae* BGR1 전체 genome에서 총 11개의 *usp* 유전자 (*usp1* 부터 *usp11*) 들을 동정하였다. 스트레스 조건에서 실험한 결과 *usp1* mutant와 *usp2* mutant들은 heat shock stress 후 1시간 내에 죽은 반면, 다른 *usp* mutants들과 wild-type인 BGR1은 45℃에서 3시간 이상 생존하였다. 모든 *usp* 유전자들의 발현은 QS에 의해 positive하게 조절되며, 직접적으로는 QsmR에 의해 조절된다. 추가적으로 *usp1*과 *usp2* 의 발현은 stationary phase에서 sigma factor인 RpoS에 의해서도 조절된다. 이것은 *usp1*과 *usp2*의 promoter region에 RpoS-RNA holoenzyme이 직접 binding하는 것으로 확인을 하였다. *usp1*의 발현은 37℃에서 28℃ 또는 45℃로 온도

shift에 의해서도 upregulation 되었다. 반면에 *usp2*의 발현은 온도 스트레스와는 무관하다. 이것은 *usp1*과 *usp2*의 발현 조절이 기존에 밝혀져 있는 *E. coli*의 기작과 다르다는 것을 나타낸다. *usp1*과 *usp2*는 heat shock stress 조건에서 *B. glumae*의 생존에 중요한 역할을 한다는 것을 확인하였다.

주요어 : *usp* gene, Quorum sensing (밀도 인식), *Burkholderia glumae*, Heat shock stress

감사의 글

지난 석박사 과정동안 많이 부족한 저를 지도해 주시고 또한 무사히 학위 과정을 마칠 수 있도록 가르침을 주신 지도교수님이신 황인규 선생님께 진심으로 깊은 감사의 말씀을 드리고 싶습니다. 심사위원장을 맡아주시고 여러 조언을 해주신 김영호 선생님과 항상 좋은 말씀을 해 주신 문제선 박사님께도 감사의 말씀을 드립니다. 대학원 과정 동안 많은 가르침을 주신 이인원 선생님, 박은우 선생님, 이용환 선생님, 가종억 선생님, 김국형 선생님과 논문심사를 맡아 주시고 격려해 주신 이상기 선생님과 김진우 박사님께도 감사 드립니다. 힘든 대학원 과정동안 저한테 많은 도움을 주신 오종희 박사님을 비롯 강용성 박사님, 최옥희 박사님께도 감사의 인사를 드립니다. 또한 묵묵히 뒤에서 조언과 충고를 아끼시지 않으셨던 안재형박사님, 이봉수박사님, 장문선박사님, 오영택박사님께도 감사의 인사를 드립니다.

지금 생각해 보면 지난 대학원 생활동안 힘들었던 일도 많았지만 나름 즐겁게 연구를 한 것 같습니다. 아마도 같은 목표를 가지고 힘든 상황 속에서도 보람과 희망을 찾는 식물세균병학 연구실 선후배님들과 함께 있었기에 그런 것 같습니다. 조금이나마 이 자리를 비로소 감사의 인사를 드립니다. 특히 대학원 과정동안 동고동락(同苦同樂)했던 훈이형과

은혜 그리고 과(科) 선후배님들께도 감사의 인사를 드립니다. 대학원 과정이라는 것이 정말 생각만큼 쉽지 않은 길이며 학문(學問)을 위해 끊임없이 자기 자신을 채찍질 하며 정진(精進)하는 것이 정말 어렵다는 것을 알았습니다. 학위(學位)라는 것이 끝이 아니며 또 다른 시작이고 자기자신이 부족하다는 것을 깨우치고 이를 위해 끊임없는 노력을 해야 한다는 것을 깨닫는 것이라고 생각합니다. 위와 같은 인고(認苦)의 과정을 통해 지금까지 헤메던 길에서 한줄기의 빛과 같은 희망과 지혜를 얻는 것이 아닐까 합니다.

마지막으로 지금까지 저를 믿고 지켜봐 주신 사랑하는 가족들 아버지, 어머니, 형, 형수님에게도 감사드리며 항상 누구보다도 저를 믿어준 나의 사랑하는 세은 그리고 뒤에서 항상 응원해 주신 아버님과 어머님에게도 마지막으로 감사의 말씀을 올리겠습니다.

2013년 1월 17일

세균병학연구실에서...