Genome-wide RNA-seq analysis of quorum sensing-dependent regulons in the plant-associated *Burkholderia glumae* strain PG1

Dissertation with the aim of achieving a doctoral degree

At the Faculty of Mathematics, Informatics and Natural Sciences

Department of Biology of the Universität of Hamburg

Submitted by

Rong Gao

From Jiangsu, China

2015 in Hamburg

Day of oral defense: 15.06.2015

The following evaluators recommend the admission of the dissertation:

Professor Dr. Wolfgang Streit

Professor Dr. Wilhelm Schäfer

Universität Hamburg
DER FORSCHUNG | DER LEHRE | DER BILDUNG

Fakultät für
Mathematik, Informatik
und Naturwissenschaften

Jun. Prof. Dr. Mirjam Perner

Molecular Biology of Microbial Consortia
Microbiology and Biotechnology
Department of Biology
Ohnhorststr. 18
D-22609 Hamburg

Tel. +49 (0)40 - 42816 -444
Fax +49 (0)40 - 42816 -459
mirjam.perner@uni-hamburg.de

16.02.2015

English language declaration

As a native English speaker, I hereby declare that I have checked the thesis "Genome-wide RNA-seq analysis of quorum sensing-dependent regulons in the plant-associated *Burkholderia glumae* strain PG1" by Rong Gao for grammatically correct English and the scientific accuracy of the language.

Sincerely,

Mirjam Perner

Abstract

Burkholderia glumae PG1 is a soil-associated motile plant pathogenic bacterium possessing a cell density-dependent regulatory mechanism called quorum sensing (QS). Its genome encodes three distinct putative autoinducer-1 synthase genes, here designated *bgaI1-3*, which are responsible for synthesizing the N-acyl-homoserine lactones (AHL). In this study, all three *bgaI* genes were characterized as the functional AHL synthase genes using AHL bioreporters *A. tumefaciens* NTL4 and *C. violaceum* CV026. To elucidate functions of these QS systems, *B. glumae* PG1 Δ*bgaI1-3* mutants were generated through allelic exchange. The deletion of each *bgaI* gene resulted in the strong reduction in motility, extracellular lipolytic activity, plant maceration and rice pathogenicity and colony morphology variation. To further identify the QS-regulated genes, the genome-wide transcriptome analysis of three Δ*bgaI* mutants vs. the parental strain was performed in the transition from exponential to stationary growth phase. In comparison with the parental strain, 481 of these genes were regulated in mutant Δ*bgaI1*, 213 in Δ*bgaI2*, and 367 in Δ*bgaI3*. In total, a set of 745 QS-regulated genes was identified. Among them, the majority of the QS-regulated genes linked to metabolic activities and the most pronounced regulation was observed for the genes involved in rhamnolipid, Flp pili, lipase, flagella, type VI secretion system and genes linked to a CRIPSR-Cas system. Notably, a cross-species analysis of QS-regulated genes revealed that *B. glumae* PG1 has more similarities with *B. thailandensis* than with the other two *Bptm* members, *B. pseudomallei* and *B. mallei* in lifestyle.

Acknowledgement

First and foremost I would like to thank my supervisor Prof. Dr. Wolfgang Streit who gave me this opportunity as a PhD student in his lab and provided me the challenging and interesting research projects and invaluable guidance for my research over the past five years and I am also grateful for his help for proofreading my thesis.

I would like to thank Prof. Dr. Wilhelm Schäfer for agreeing to be my second superviser and Jun. Prof. Dr. Mirjam Perner for being a language proofreader of my thesis.

I would like to thank Sabine Zumbraegel for supervising me laboratory techniques and Dr. Claudia Katharina Hornung for sharing her microbiological knowledge with me, Nicolas Rychlik for providing the vector pNPTS138-R6KT, Dr. Jennifer Chow for providing the control strain of the lipase test and Angela Jordan for her input in the TLC assay, Elke Woelken and PD Dr. Eva Spieck for their help in the TEM expriments, Marion Klötzl for the assistance with growing the rice plant; Dr. Andreas Knapp for the bioinformatics analysis of CRISPR and T6SS.

I would like to thank Dr. Yuchen Han and Jiajia Tang for proofreading my thesis and their scientific suggestions.

I would like to thank all people who contributed to the transcriptome publication based on this work: Dr. Dagmar Krysciak for proofreading my publication, Christian Utpatel for producing my transcriptome circular maps, Dr. Christel Schmeisser for her scientic suggestions of mutation technique, Prof. Rolf Daniel and Dr. Sonja Voget for their RNA-seq service from the Göttingen Genomics Laboratory of Georg-August-Universität Göttingen, and Dr. Andreas Knapp and Prof. Karl-Erich Jaeger for their assistance with the qPCR experiments from the institute of molecular enzymtechnology of Heinrich Heine University Düsseldorf, Prof. Dr. Wolfgang Streit.

I would like to thank all my colleagues: Andreas, Angela, Arek, Andrea, Boris, Birhanu, Christel, Christian, Christiane, Dagmar, Dominik, Ebrahim, Gabi, Gesche, Han, Hanae, Henning, Ines, Janine, Jessica, Jenny C., Jenny H., Joana, Julia, Jeremy, Katja, Maria, Mariita, Martina, Mirjam, Moritz, Nele, Nico R., Nico A., Norwin, Patrick, Philipp, Regina, Sabine K., Sabine Z., Sascha, Steffi, Simon, Simone, Tanja, Ulrich, Uschi and Wenke. Thanks for your help and supports.

I would like to thank my country and Chinese Scholarship Council for subsidizing me to study in Germany.

Special thanks to my parents and my wife for their unselfish supports and love.

At last, **many many many thanks** for the people who helped me and supported me**!!!**

The results of this thesis were published as follows:

Gao R, Krysciak D, Utpatel C, Knapp A, Schmeisser C, Daniel R, Voget S, Jaeger KE, Streit WR. 2015. Genome-wide RNA sequencing analysis of quorum sensing-controlled regulons in the plant-associated *Burkholderia glumae* strain PG1. Appl Environ Microbiol., *accepted*.

Knapp A, Voget S, **Gao R**, Zaburannyi N, Poehlein A, Krysciak D, Breuer M, Hauer B, Streit WR, Müller R, Daniel R, Jaeger KE. 2015. Biotechnological potential of the lipase producing strain *Burkholderia glumae* PG1. Appl Microbiol Biotechnol., *Submitted*.

Table of Contents

Table of Figures

Table of tables

I. Introduction

1. Quorum sensing

Quorum sensing (QS) is a cell to cell communication process based on population density, which is employed by a wide variety of bacteria to regulate their group behavior in order to adaptation to the environment (Waters & Bassler, 2005) (Figure 1). This process is dependent on the production, release, and group-wide detection of some kinds of signal molecules called autoinducers (AIs) by bacteria (Fuqua & Winans, 1994; Jayaraman & Wood, 2008; O'Loughlin *et al.*, 2013). A critical threshold concentration of AIs can be reached with increasing of population density, which triggers the expression of related genes, resulting in changes of bacterial life cycle and metabolism (Waters & Bassler, 2005). It is known that QS regulates a variety of complex activities, such as antibiotics production, root nodulation, sporulation, bioluminescence, pigment production, competence for DNA uptake, motility, virulence factors secretion, and biofilm formation in diverse bacteria (Fuqua & Greenberg, 2002; Ng & Bassler, 2009; Waters & Bassler, 2005).

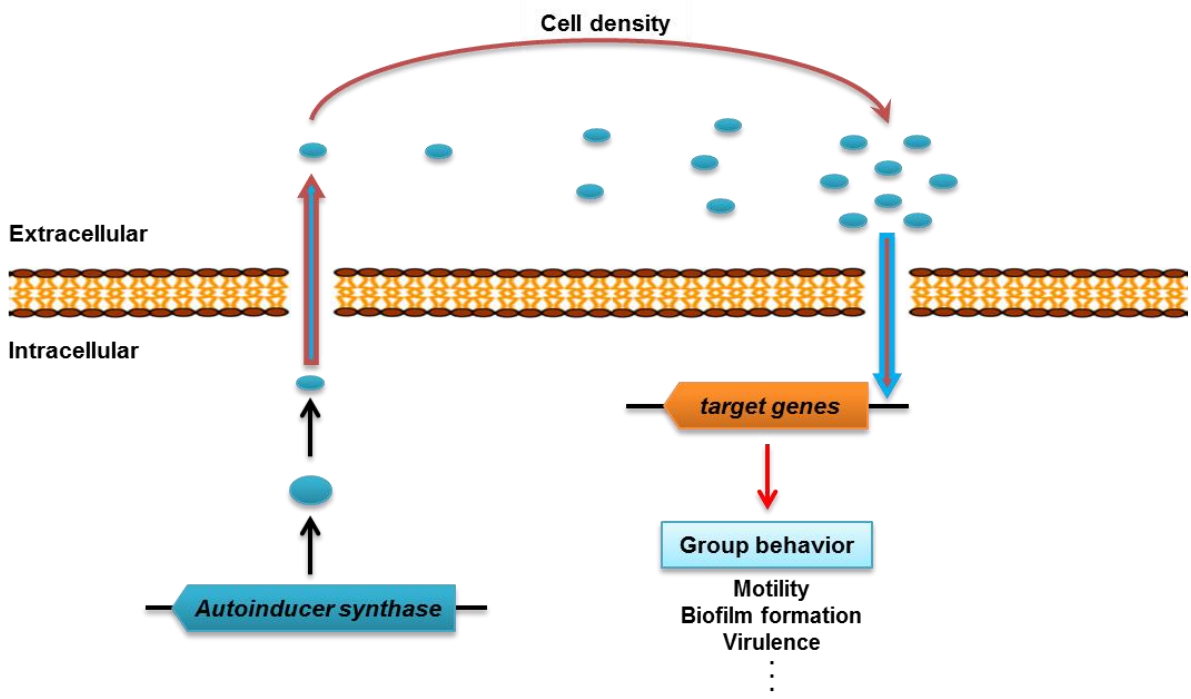


Figure 1. Bacterial quorum sensing. Quorum sensing is a cell to cell communication process which is mediated by different kinds of signal molecules, called autoinducers (AIs). After synthesis, AIs are released out of cells by diffusion or with the help of transporters. When cell density reaches a threshold,

AIs can enter cells again and bind to their regulators to regulate gene expression or directly use the two-component signal transduction system to trigger the gene regulation. Both in the end could result in changes of bacteria group behavior, such as motility, biofilm formation, virulence, and so on.

1.1. Classification of QS systems

In the last few decades, it was identified that a large number of bacteria species employ QS regulatory circuits to coordinate group behaviors. Although there are variations between different species in terms of AIs and mechanisms of signal transduction, the majority of identified QS systems can be classified into several categories based on the common structural features of AIs.

1.1.1. AHL-based QS systems in Gram-negative bacteria

N-acyl-homoserine lactone (AHL) based QS system used by Gram-negative bacteria is the most intensely investigated system at the molecular level (Figure 2A). AHL, also known as autoinducer-1 (AI-1), was the first described AI molecule by J. Woodland Hastings and colleagues in 1979. They found that the bioluminescent marine bacterium *Vibrio fischeri* employs N-3-oxo-hexanoyl homoserine lactone (3-oxo-C6-AHL) (Figure 2A) as a signaling molecule to modulate bioluminescence (Eberhard *et al.*, 1981). Seven luminescence genes (*lux*) were identified in two transcriptional units in this bacterium. One unit comprises the *luxR* gene, while the other is an operon including *luxI* and *luxCDABEG* genes required for light production. LuxI is responsible for synthesizing of 3-oxo-C6-AHL, and LuxR is the cognate receptor of 3-oxo-C6-AHL (Engebrecht & Silverman, 1984), which is unstable and degraded rapidly in the absence of 3-oxo-C6-AHL. In a low population density, 3-oxo-C6-AHL diffuses into and out of cells and keeps it in a low concentration to sustain a “turn off” state. While with the increase of population density, a threshold concentration can be achieved and allows it binds to LuxR and activates it by exposing a DNA binding domain (Zhang *et al.*, 2002; Zhu & Winans, 2001). Then the DNA binding domain binds to a 20 bp sequence in the 42.5 bp upstream of the *luxI* promoter start site and activates transcription of the *luxICDABEG* operon (Hanzelka & Greenberg, 1995; Kaplan & Greenberg, 1985; Stevens *et al.*, 1994). The *luxA* and *luxB* genes encode for α and β subunits respectively of the luciferase enzyme which is able to catalyze the oxidation reaction to produce bioluminescence. And the *luxCDEG*

encodes for the fatty acid reductase complex which produces and recycles luciferase aldehyde substrates to enhance bioluminescence (Lupp *et al.*, 2003; Nijvipakul *et al.*, 2008).

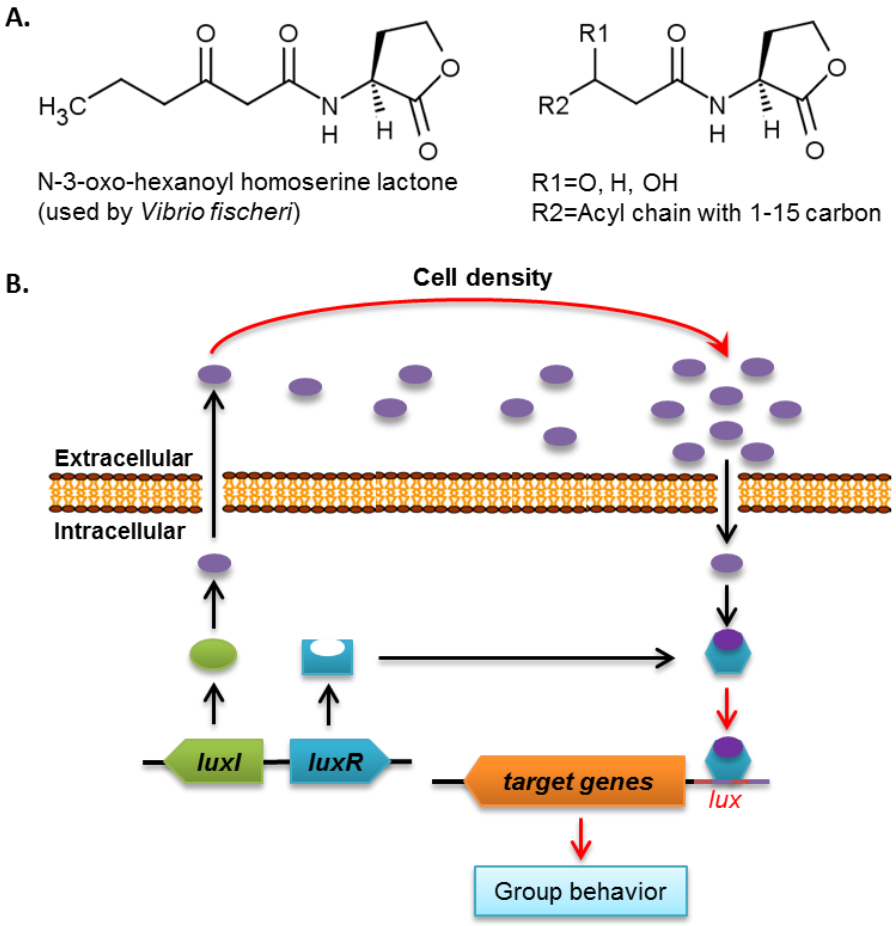


Figure 2. AHL-based QS in Gram-negative bacteria. **(A)** Mechanism of AHL-based QS in Gram-negative bacteria. AHLs catalyzed by LuxI-like protein freely diffuse into or out of the cell. At high cell density, AHLs in cell bind to the transcriptional regulator LuxR and regulate the transcription of target genes. **(B)** Chemical structure of AHL and the first identified AHL molecule from *Vibrio fischeri*.

Since this first description of the AHL-mediated QS system, many homologous of *luxIR* genes have been identified in more than 100 species of Gram-negative bacteria. And their QS functions have been demonstrated in most species (Hirakawa & Tomita, 2013; Manefield *et al.*, 2002). Almost all of them employ the similar regulation mechanism (Figure 2B) as in *Vibrio fischeri* to monitor their population density and coordinate group behaviors. Additionally, some bacteria produce two or more different AHL molecules and different bacteria can synthesize the same AHL molecule. Hence, there are some overlaps in the production and recognition of AHLs by different bacteria which indicates the possibility of a crosstalk between different Gram-negative bacteria species (Table 1).

Table 1. Examples of AHL-mediated QS in Gram-negative bacteria.

Bacterium	LuxR/I	AHL	Reference
A. hydrophila	AhyIR	C4-AHL	(Swift et al. 1999)
A. salmonicida	AsaIR	C4-AHL	(Mcclean et al. 1997)
A. tumefaciens	TraIR	OC8-AHL	(Zhu et al. 1998)
B. thailandensis	BtaIR1 BtaIR2 BtaIR3	C8-AHL OHC10-AHL OHC8-AHL	(Majerczyk et al., 2014a)
B. cepacia	CepIR	C8-AHL	(Lewenza et al. 1999)
B. glumae	TofIR	C6-,C8-AHL	(Kim et al. 2004)
C. violaceum	CviIR	C6-AHL	(Chernin et al. 1998)
E. agglomerans	EagIR	OC6-AHL	(Swift et al. 1993)
E. chrysanthemi	ExpIR	OC6-AHL	(Nasser et al. 1998)
N. multiformis	NmuIR	C14-,OC14-AHL	(Gao et al. 2014)
P. stewartii	EsaIR	OC6-AHL	(Minogue *et al.*, 2005)
P. aeruginosa	LasIR RhlIR	OC12-AHL C4-AHL	(Pearson *et al.*, 1997)
P. aureofaciens	PhzIR	C6-AHL	(Wood et al. 1997)
R. solanacearum	SolIR	C8-AHL	(Flavier et al. 1997)
R. leguminosarum	CniIR RaiIR RhiIR TraIR	7-cis-OHC14-AHL C8-,OHC8-AHL C6-,C7-,C8-AHL C8-,OC8-AHL	(Jones et al. 2002) (Rodelas et al. 1999)
S. liquefaciens	SwrIR	C4-AHL	(Givskov et al. 1998)
V. fischeri	LuxIR	OC6-AHL	(A Eberhard et al. 1981)
Y. enterocolitica	YenIR	C6-AHL	(Throup et al. 1995)

1.1.2 Oligopeptide-mediated QS system in Gram-positive bacteria

Instead of using AHL to mediate QS by Gram-negative bacteria, Gram-positive bacteria utilize post-transnationally modified oligopeptides as AIs, designated as autoinducing peptides (AIPs) (Ng & Bassler, 2009). AIPs-based system employs a two-component regulatory signal transduction system, including a histidine protein kinase (HPK) in cell membrane and an

intracellular response regulator (RR), to sense signal molecules and induce cellular responses (Figure 3A). AIPs are produced in the cell and secreted out of cells with the help of transporters because the cell membrane is impermeable to oligopeptides. At the threshold concentration, the mature AIPs bind to the N-terminal domain of HPK, resulting in activation of HPK by phosphorylation of a conserved histidine residue of it in cytoplasm. Then, the phosphoryl group of the HPK is transferred to an intracellular RR, which ultimately functions as DNA binding transcription factor to regulate the transcription of target genes (Dobson *et al.*, 2012; Sturme *et al.*, 2002).

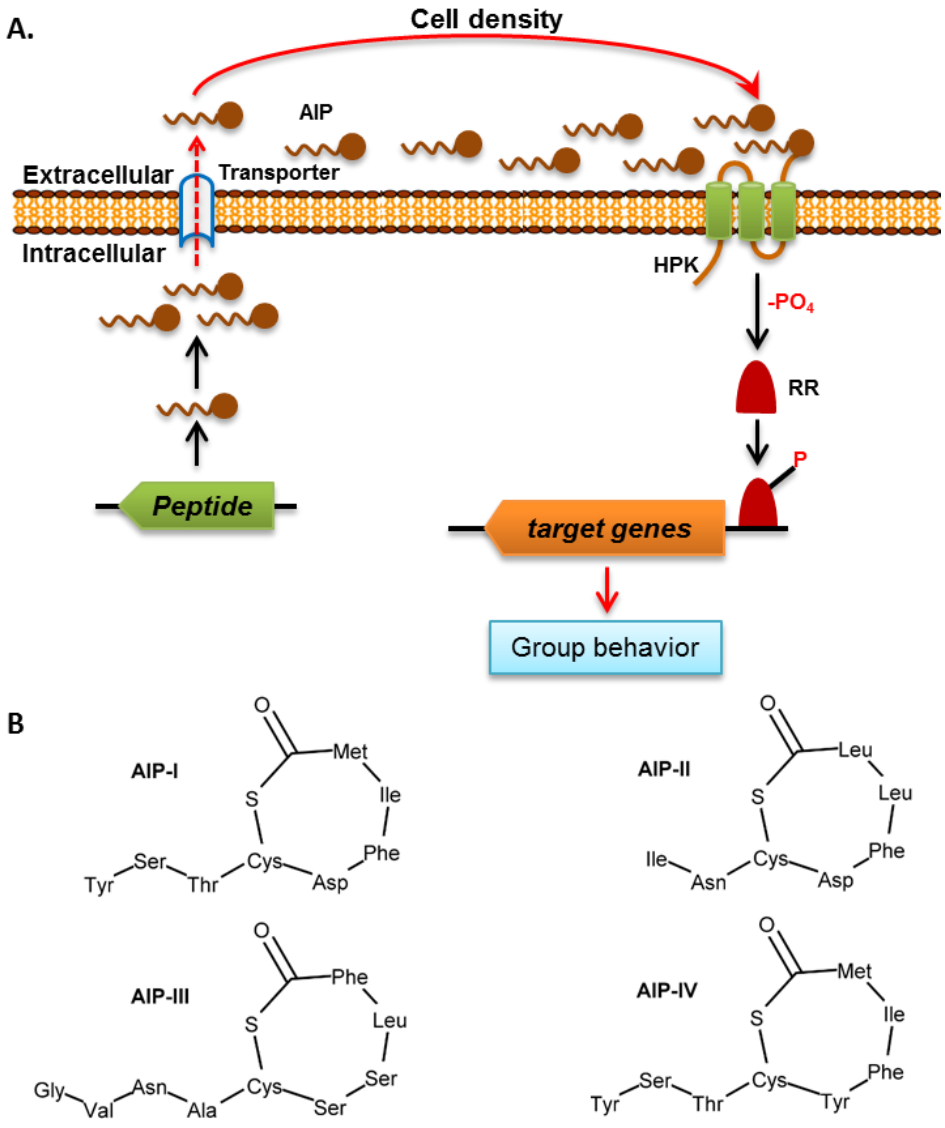


Figure 3. Oligopeptide-based QS in Gram-positive bacteria. (A) Mechanism of Oligopeptide-based QS in Gram-positive bacteria. Autoinducer oligopeptides (AIPs) are exported to cells and bind to cell surface-bound sites to activate phosphorylation cascades, resulting in regulating the transcription of target genes. **(B)** Structures of the AIPs used by *S. aureus*.

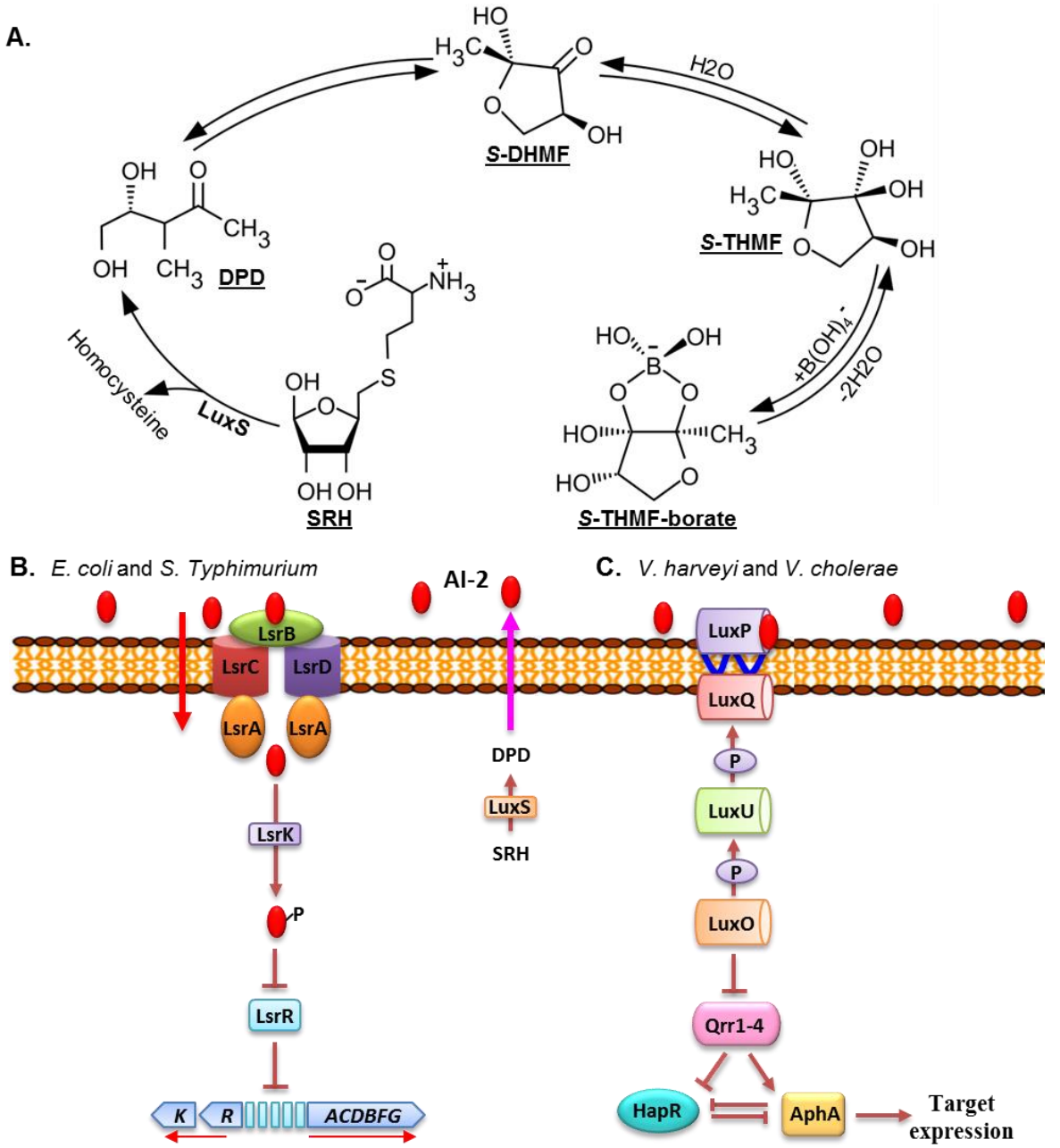


Figure 4. AI-2-based QS in Gram-negative and Gram-positive bacteria. (A) Biosynthesis process of AI-2 catalyzed by *S*-ribosylhomocysteinase (LuxS). SRH is converted to homocysteine and 4,5-dihydroxy-2,3-pentanedione (DPD) under catalyzing of LuxS. Then DPD spontaneously cyclizes to form the furanone and act as AI-2 for *Salmonella typhimurium* and *E. coli* or further interacts with borate to form a furanosyl borate diester act as another type of AI-2 for *Vibrio harveyi* and *Vibrio cholera*. **(B)** In *Salmonella typhimurium* and *Escherichia coli*, AI-2 combines with periplasmic receptor LsrB and is transported into cell by the LsrABCD transport system. And then, AI-2 is phosphorylated by LsrK and presumed to interact with LsrR. After LsrR removed, the transcription of *lsr* operon is activated. **(C)** In *Vibrio harveyi* and *Vibrio cholera*, AI-2 interacts with LuxP in the periplasm and initiates the LuxQ/LuxO phoshporyl cascade in high cell density. The dephosphorylation of LuxO results in the repression of Qrr gene expression and increase of HapR expression. HapR represses *aphA* to regulate expression of target genes.

Most functional AIPs are produced from segmenting of larger precursor peptides, followed by linearization, cyclization or modifications with substitutions of isoprenyl groups to form lactone and thiolactone rings (Ansaldi *et al.*, 2002; Rutherford & Bassler, 2012). Each AIP has a typical chemical architecture based on the length and post-translational modifications, which confers it a high level of selectivity and specificity (Figure 3B). AIPs range in size from 5 to 17 amino acids and have been classified into three categories according to characteristics of their structures (Ahmad *et al.*, 2011). The first category is called oligopeptide lantibiotics. Peptides in this class contain characteristic polycyclic thioether amino acids lanthionine or methyllanthionine, as well as unsaturated amino acids dehydroalanine and 2-aminoisobutyric acid (Quadri, 2002). The second one is termed as the 16-membered side-chain-to-tail thiolactone peptide, typified by the modified octapeptide AIP-1 from *Staphylococcus aureus* (Scott *et al.*, 2003). The third one is the isoprenylated tryptophan peptides, which are produced by isoprenylation of inactive precursor peptides. Their precise structures are unknown so far (Ahmad *et al.*, 2011).

1.1.3. Autoinducer-2-based QS systems in Gram-negative and Gram-positive bacteria

The autoinducer-2 (AI-2) mediated QS system was first described in the Gram-negative bacterium *V. harveyi* (Bassler *et al.*, 1994). Since it is able to be used by both Gram-negative and Gram-positive bacteria, it is also designated as “universal QS”. AI-2 is produced from *S*-ribosylhomocysteine (SRH) catalyzed by LuxS protein, whose homologous can be found in 537 of the 1402 bacterial genomes currently sequenced (Pereira *et al.*, 2013). LuxS can convert SRH to (*S*)-4, 5-dihydroxy-2, 3-pentanedione (DPD) which cyclizes spontaneously to form AI-2 (Figure 4A). Due to the natural property of being hydrophilic and low affinity for lipid binding, AI-2 is transported out of the cells with the help of carrier proteins. After accumulating to a threshold concentration in the extracellular with the increasing population density, AI-2 is transported into the cells by special routes (Pereira *et al.*, 2013).

Currently, two such routes have been identified. In *E. coli* and *S. typhimurium*, AI-2 is imported into the cells with the help of Lsr transporter encoded by the first four genes of *lsrACDBFG* operon. Then, it is phosphorylated by LsrK in the cytoplasm and binds to the LsrR repressor

which inactivates LsrR to derepression of transcription of *lsrACDBFG* (Xavier & Bassler, 2005) (Figure 4B). LsrG and LsrF are both involved in the further processing of phosphorylated AI-2, although their function are not clear so far (Xavier *et al.*, 2007). The second route was found in *V. harveyi* and *V. cholera* (Figure 4C). Different from the transporter system, this route is formed by a series phosphorylation signaling cascades which mainly regulated by the binding protein LuxP, the sensor protein LuxQ and LuxU (Ng & Bassler, 2009). In a high cell density, extracelluar AI-2 binds to LuxP in the periplasm to reverse the phosphate fluxes pathway and then switches the activity of LuxO, which is located in the cytoplasm, from kinase to phosphatase. The dephosphorylated LuxO loses the ability to induce transcription of Qrr1-4, resulting in down regulation of low cell density regulator AphA (O'Loughlin *et al.*, 2013) and activation of the expression of HapR, which shut off the production of virulence factor. This type of AI-2 QS system existing in *Vibrio* species regulates the expression of more than 100 genes (Rutherford & Bassler, 2012; Waters & Bassler, 2006).

1.1.4. Additional QS systems

Besides AHL, AIP and AI-2, some other AIs are also discovered and employed by bacteria to regulate bacterial QS-dependent behaviors. For example, 2-heptyl-3hydroxy-4 quinolone, called *Pseudomonas* quinolone signal (PQS) (Figure 5A), acts as an AI in *Pseudomonas* to control expression of a series of genes for virulence and biofilm formation (Diggle *et al.*, 2007). Diffusible signal factor (DSF) was discovered to function as an AI in *Xanthomonas campestris*, *X. oryzae*, *Xylella fastidiosa* and *Stenotrophomonas maltophilia* (Qian *et al.*, 2013) (Figure 5B-C). *Burkholderia* diffusible signal factor (BDSF), similar as DSF, was recently described in *Burkholderia cenocepacia* and functions for interspecies communications (Udine *et al.*, 2013) (Figure 5D). 3-hydroxypalmitic acid methyl ester (3-OH PAME), produced by the plant pathogen *R. solanacearum*, functions as an AI by regulating its virulence factors in a population dependent manner (Clough *et al.*, 1997) (Figure 5E). Recently, a novel AI molecule, the α-hydroxyketones (AHKs), was characterized in the Gram-negative bacteria *L.pneumophila* and *Vibrio* spp (Miller *et al.*, 2002; Tiaden *et al.*, 2010a) (Figure 5F-G). With further research, it is undisputed that more AIs will be discovered and investigated.

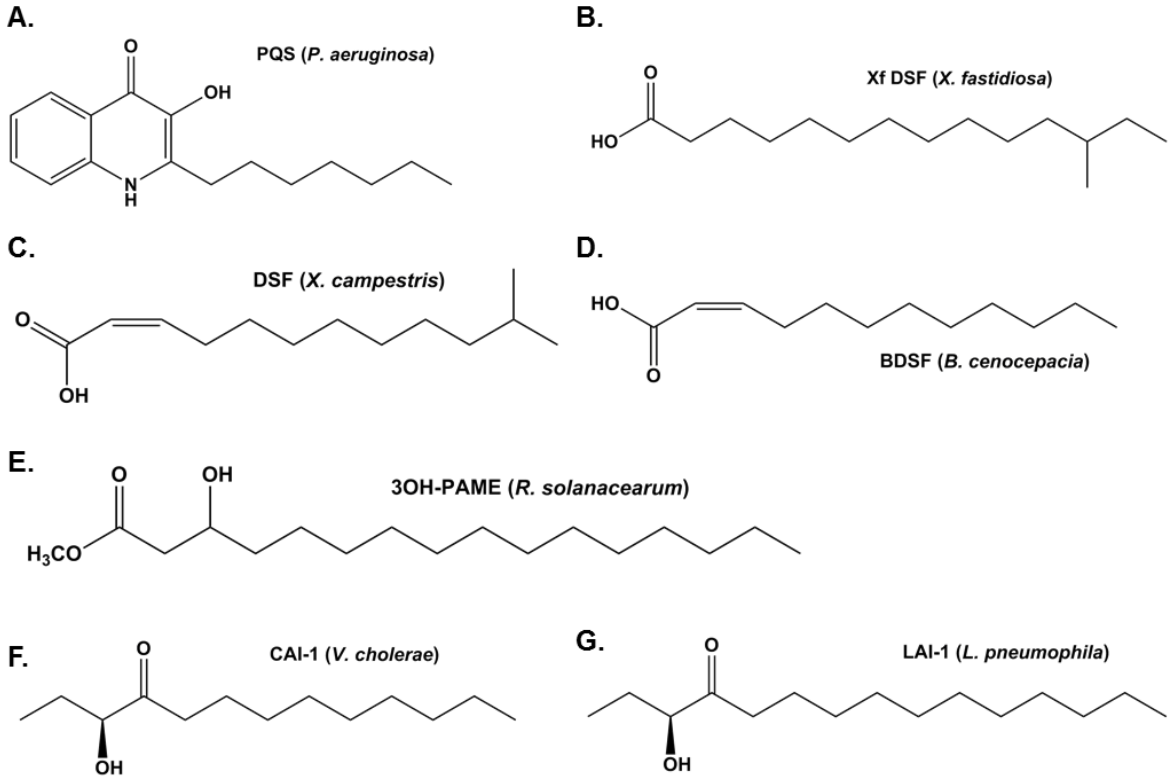


Figure 5. Structure of other QS signal molecules used by bacteria. **(A)** PQS, *Pseudomonas* quinolone signal. **(B-D)** DSF, diffusible signal factor. **(E)** 3OH-PAME, 3-hydroxypalmitic acid methyl ester. **(F)** CAI-1, (*S*)-3-hydroxytridecan-4-one. **(G)** LAI-1, (*S*)-3-hydroxypentadecan-4-one.

1.2. N-acyl-homoserine lactone (AHL)

1.2.1. Structure of AHL

AHLs are produced and employed by a large number of Gram-negative bacterial species to regulate the expression of functional genes in a cell density-dependent manner. The first AHL molecule was found in *Vibrio fischeri* in1981. From then till now, dozens of AHLs have been identified and almost all of them are composed by one conserved homoserine lactone ring and one acyl chain with variable length depending on bacteria species (Williams, 2007). The real role of each part of AHL is poorly defined. However, it is hypothesized that the amphipathic structure could facilitate AHLs to navigate the phospholipid bilayer of cell membranes as well as aquiferous endocellular and extracellular environments (Greenberg, 1994). The acyl chain can be modified by a 3-oxo substituent, a 3-hydroxyl substituent and a terminal methyl branch, or varied degrees of unsaturation (Fuqua & Greenberg, 2002)(Figure 6A).

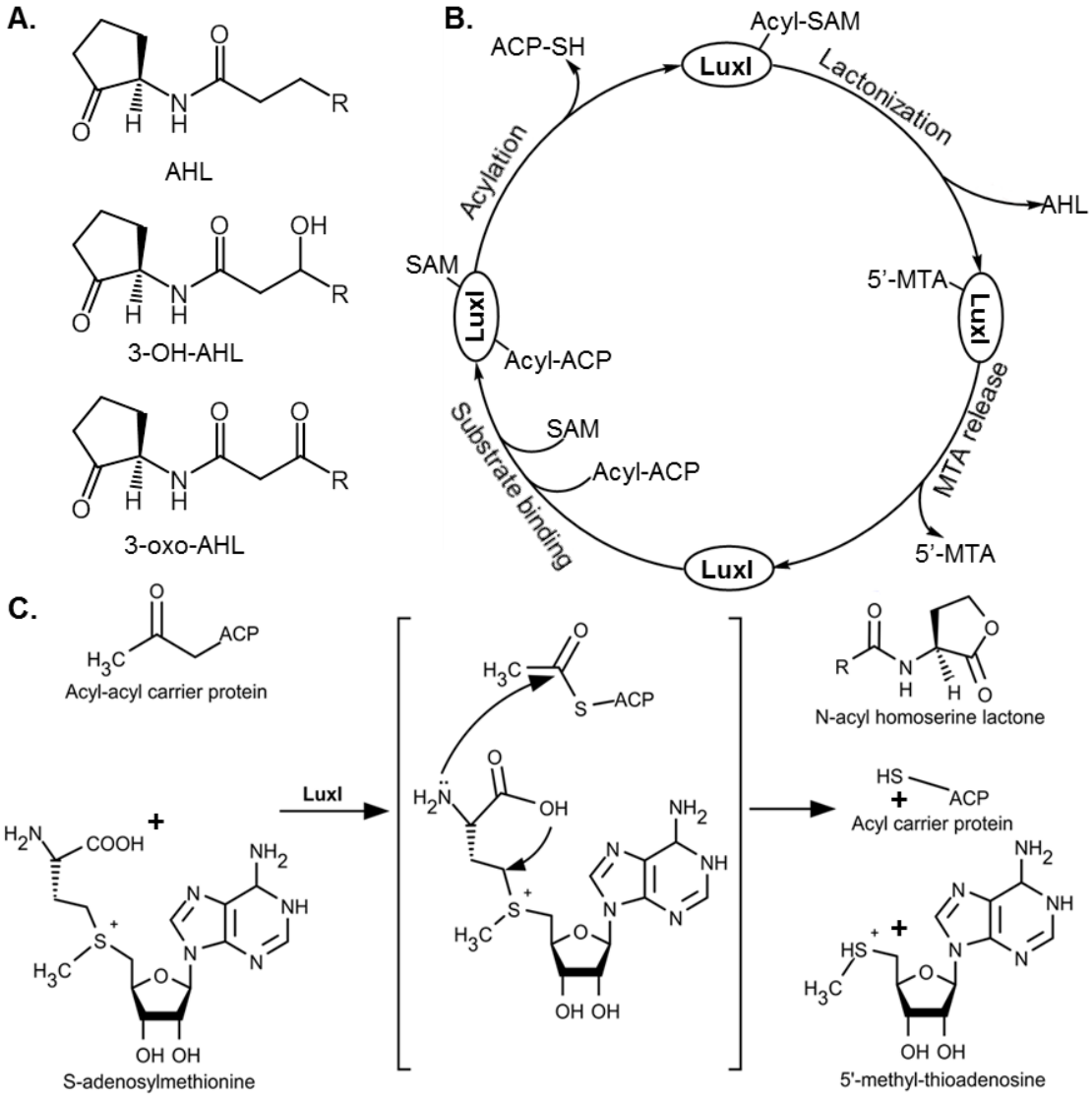


Figure 6. Categories and biosynthesis of AHLs. (A) AHLs are broadly classified into three groups based on the substitution in the acyl moiety: AHL, 3-hydroxyl-AHL and 3-oxo-AHL. **(B-C)** Biosynthesis of AHLs catalyzed by LuxI family. SAM and acyl-ACPs are substrates, and acylation of the amino group of SAM and lactonization of the acyl-SAM intermediate are two reactions required for AHLs production. SAM, *S*-adenosylmethionine; ACP, acyl carrier protein; MTA, methylthioadenosine.

1.2.2. Synthesis process of AHL

There are three protein families are known to be able to synthesize AHL molecules. The first and mainly employed synthase is the LuxI type family, which use substrates of *S*-adenosylmethionine (SAM) and acylated acyl-carrier protein from lipid metabolism to produce AHL molecules (Parsek *et al.*, 1999). Members in this family are in different in size, 190–230 amino acids in length and share 4 blocks of conserved sequence domains. And amino acid sequence alignments revealed that 10 amino acids are completely conserved

within LuxI family (Pappas *et al.*, 2004). Studies on the crystal structure indicated that LuxI type synthases share structural similarity with the N-acetyltransferases (Watson *et al.*, 2002). The second family was only found in Vibrio species, including LuxM from *Vibrio harveyi*, AinS from *Vibrio fischeri* and VanM from *Vibrio anguillarum* (Fuqua & Greenberg, 2002). These synthases are responsible for synthesizing 3-OH-C4-AHL, C8-AHL and 3-OH-C6-AHL, respectively. Even though they synthesize AHLs by the same mechanism as LuxI family, they show none sequence similarity to them. The third family includes HdtS in *Pseudomonas fluorescens* and Act in *Acidithiobacillus ferrooxidans*, which are bound up with the lysophosphatidic acid acyltransferase protein family (Laue *et al.*, 2000; Rivas *et al.*, 2007). The HdtS type synthases have been found in only a few bacterial species and mechanism of these synthases remains to be unclear. In *Pseudomonas fluorescens,* HdtS directs the synthesis of three different AHLs: N-(3-hydroxy-7-cis-tetradecenoyl)-homoserine lactone (3-OH-C14:1-AHL), N-hexanoyl-homoserine lactone (C6-HSL) and N-decanoyl-homoserine lactone (C10-AHL), and in *Acidithiobacillus ferrooxidans* Act directs synthesis of N-tetradecanoylhomoserine lactone (C14-HSL) together with small amounts of shorter-chain AHLs.

In principle, the synthesis of a single AHL is only catalyzed by a single synthase. In 1991, Eberhard *et al*. used crude cell lysate of *V. fischeri* to perform AHL synthesis assay in vitro and firstly demonstrated that SAM and 3-oxohexanoyl coenzyme A (3-oxohexanoyl CoA) are substrates for AHL synthesis in *V. fischeri* (Eberhard *et al.*, 1991). In 1996, More *et al*. purified TraI protein from *E. coli* containing the traI gene from *A. Tumefaciens*. And they identified that TraI used SAM to synthesize homoserine lactone moiety and used 3-oxo-octanoyl-acyl carrier protein (3-oxo-octanoyl ACP) rather than 3-oxo-octanoyl CoA to synthesize the 3-oxo-octanoyl moiety (Moré *et al.*, 1996). Subsequently, the studies of Schaefer' group and Jiang's group confirmed the finding of More and his associates with the purified LuxI protein of *V. fischeri* and the purified RhlI protein of *P. aeruginosa*, respectively (Jiang *et al.*, 1998; Schaefer *et al.*, 1996). And laboratory studies of Parsek's group have further confirmed this opinion with the purified RhlI protein overexpressed in its native environment, *P. aeruginosa* (Parsek *et al.*, 1999).

The synthesis of AHL from SAM requires two steps: acylation of the amino group of SAM and lactonization of the acyl-SAM intermediate (Figure 6B-C) (Fast & Tipton, 2012). The first step is the AHL synthase combining with the substrates, SAM and acyl-ACP. SAM is a common precursor which can be recognized by most of AHL synthases, including the first and second families of AHL synthases, but acyl-ACP only can be recognized by specific bacteria depending on the features of their synthases (Parsek *et al.*, 1999). Second, under catalysis of AHL synthase, the cyclization occurs with a nucleophilic attack on the carbonyl position of C1 by the amino nitrogen of SAM, producing an amide bond. And this is coupled to the release of by-products ACP-SH (Parsek *et al.*, 1999). Subsequently, the lactonization is accomplished with another nucleophilic attack on the carbon of SAM by its own carboxylate oxygen resulting in the formation of homoserine lactone ring. Finally, one molecule of AHL is released from AHL synthase with concomitant expulsion of *S*-methylthioadenosine (MTA), which is the final product and can initiate a new round of synthesis (Parsek *et al.*, 1999).

2. The genus *Burkholderia* and its QS systems

2.1 The genus *Burkholderia*

Based on 16S rRNA sequences, DNA-DNA hybridization, fatty acid analysis, and several phenotypic characteristics, the RNA homology group II of the genus *Pseudomonas* was renamed as the genus *Burkhoderia*, including seven different species: *B. solanacearum*, *B. pickettii*, *B. cepacia*, *B. gladioli*, *B.mallei*, *B. pseudomallei*, *B.caryophylli* (Yabuuchi *et al.*, 1992). And *Pseudomonas pickettii* and *Pseudomonas solanacearum* were classified into the genus *Ralstonia* (Yabuuchi *et al.*, 1992).

The genus *Burkholderia* is rod-shaped, motile, Gram-negative bacterium within the class of the β-proteobacteria, and capable of degrading a diverse viariety of carbon compounds as carbon source (Master & Mohn, 1998). Since the first *Burkholderia* species published by Walter H. Burkholder in 1942 which later were named as *Burkholderia caryophylli* (*Pseudomonas caryophylli*) and Burkholderia pv. *allicola* (*Pseudomonas allicola*), more than 80 species of *Burkholderia* were isolated currently (Estrada-de los Santos *et al.*, 2013). Due to their remarkable metabolic versatility, *Burkholderia* species can thrive in surprisingly diverse ecological niches including water, soil, sediments, even in some extreme environments:

nutrient limitation, antibiotics and toxic (Figure 7). This species can parasitize a diverse array of hosts, including plants, animals, human, insects and fungi, and induce pathogenicity in hosts or lead a symbiosis with hosts (Figure 7) (Coenye & Vandamme, 2003).

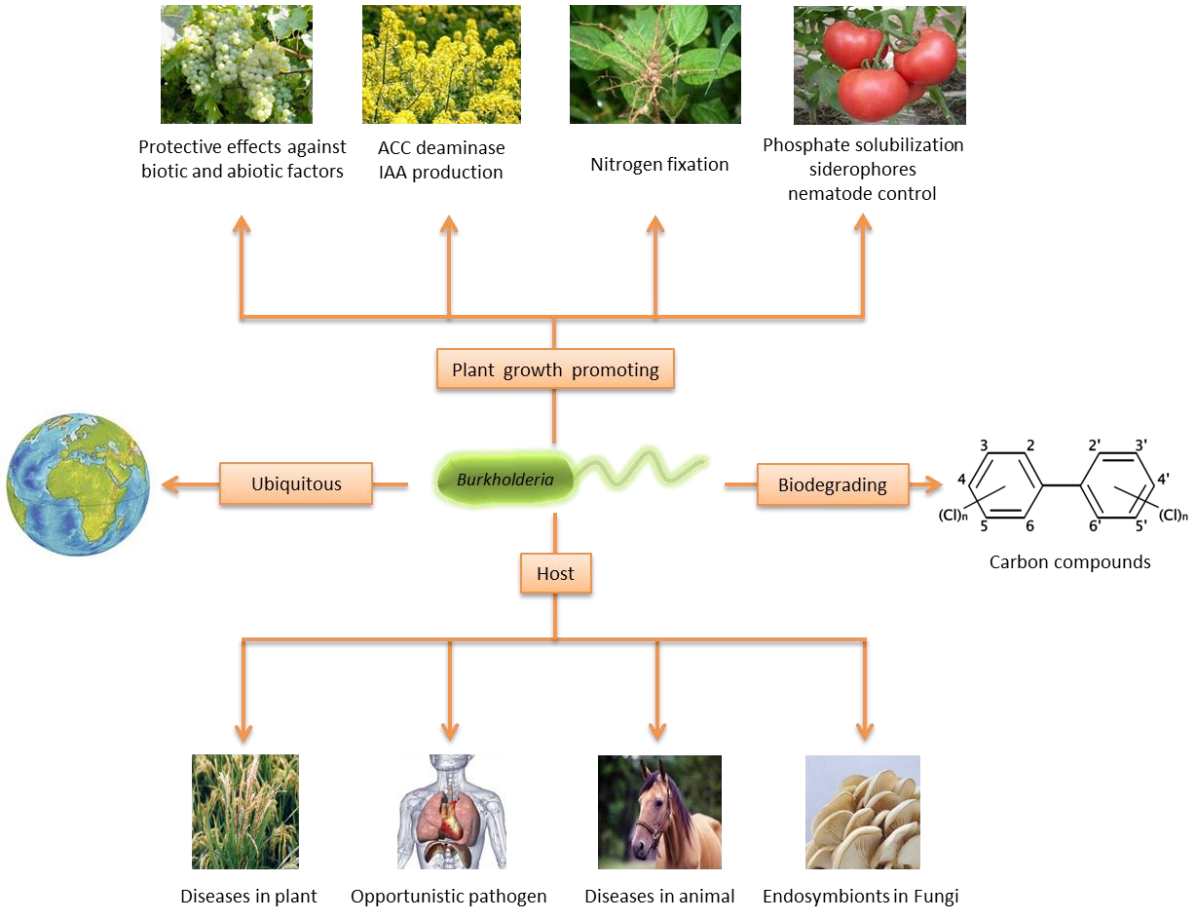


Figure 7. Lifestyle of the genus *Burkholderia*.

2.1.1. Taxonomy of the genus *Burkholderia*

The genus *Burkholderia* can be divided into two main clusters based on results of phylogenetic analysis on 16S rRNA, *recA*, *gyrB*, *rpoB* and *acdS* (Figure 8)(Estrada-de los Santos *et al.*, 2013; Suárez-Moreno *et al.*, 2012). In the first cluster, most of strains are pathogenic to plant, animal or human, including *B. cepacia* complex (BCC), *Bptm* group and *Pseudomallei* group. Some other species that are endosymbionts in phytopathogenic fungi also belong to this cluster. The second cluster can be further classified into two sub-groups. One is the plant-associated beneficial species, which can either promote the growth of plant or enhance nutrient-uptake of plant. Another sub-group mainly contains saprophytic species, which has the ability to degrade aromatic compounds.

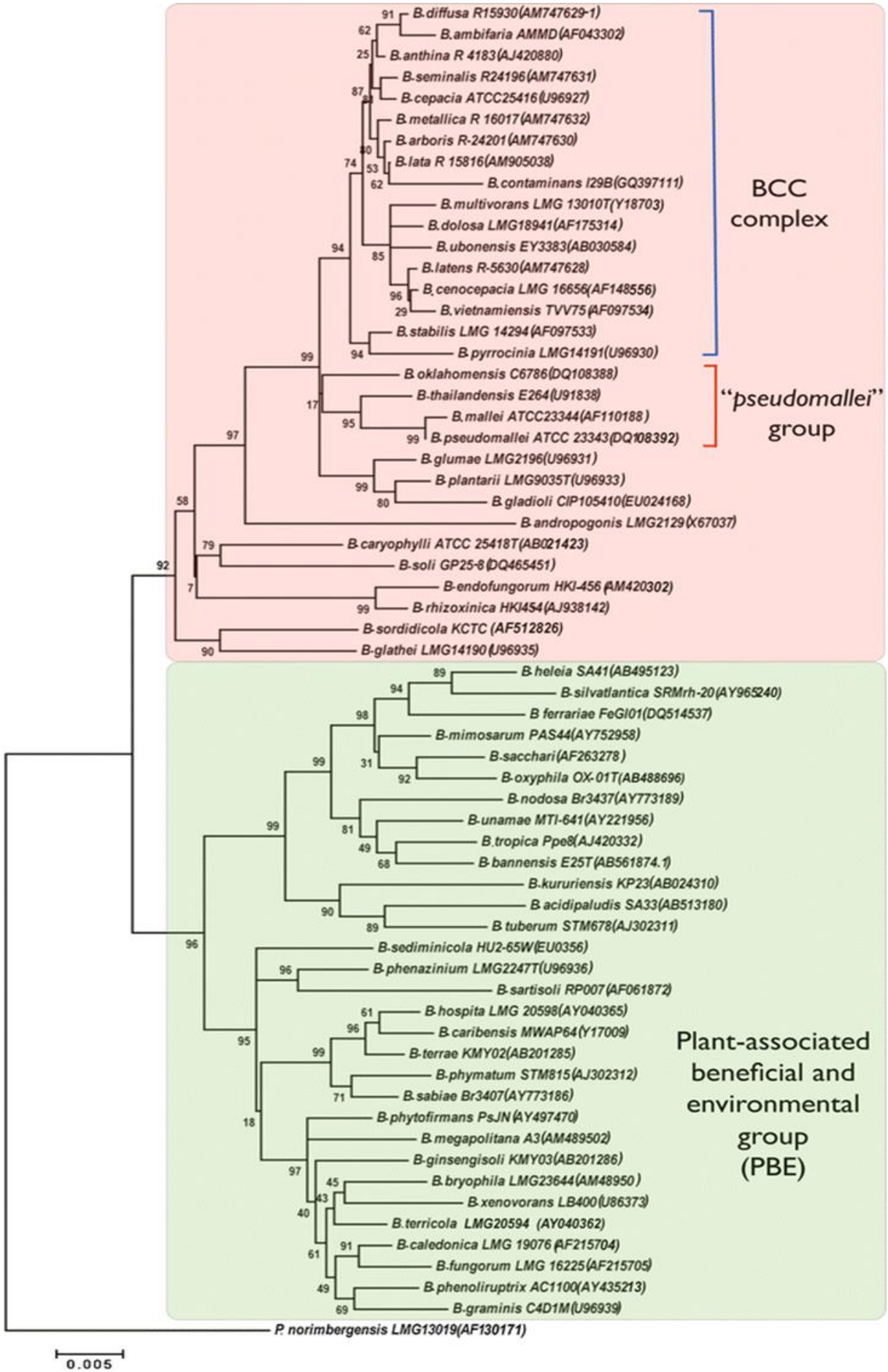

B.diffusa R15930(AM747629-1)
B.ambifaria AMMD(AF043302)
B.anthina R 4183(AJ420880)
B.seminalis R24196(AM747631)
B.cepacia ATCC25416(U96927)
B.metallica R 16017(AM747632)
B.arboris R-24201(AM747630)
B.lata R 15816(AM905038)
B.contaminans I29B(GQ397111)
B.multivorans LMG 13010T(Y18703)
B.dolosa LMG18941(AF175314)
B.ubonensis EY3383(AB030584)
B.latens R-5630(AM747628)
B.cenocepacia LMG 16656(AF148556)
B.vietnamiensis TVV75(AF097534)
B.stabilis LMG 14294(AF097533)
B.pyrrocinia LMG14191(U96930)
BCC complex
B.oklahomensis C6786(DQ108388)
B.thailandensis E264(U91838)
B.mallei ATCC23344(AF110188)
B.pseudomallei ATCC 23343(DQ108392)
"pseudomallei" group
B.glumae LMG2196(U96931)
B.plantarii LMG9035T(U96933)
B.gladioli CIP105410(EU024168)
B.andropogonis LMG2129(X67037)
B.caryophylli ATCC 25418T(AB021423)
B.soli GP25-8(DQ465451)
B.endofungorum HKI-456(AM420302)
B.rhizoxinica HKI454(AJ938142)
B.sordidicola KCTC (AF512826)
B.glathei LMG14190(U96935)
B.heleia SA41(AB495123)
B.silvatlantica SRMrh-20(AY965240)
B ferrariae FeGl01(DQ514537)
B.mimosarum PAS44(AY752958)
B.sacchari(AF263278)
B.oxyphila OX-01T(AB488696)
B.nodosa Br3437(AY773189)
B.unamae MTI-641(AY221956)
B.tropica Ppe8(AJ420332)
B.bannensis E25T(AB561874.1)
B.kururiensis KP23(AB024310)
B.acidipaludis SA33(AB513180)
B.tuberum STM678(AJ302311)
B.sediminicola HU2-65W(EU0356)
B.phenazinium LMG2247T(U96936)
B.sartisoli RP007(AF061872)
B.hospita LMG 20598(AY040365)
B.caribensis MWAP64(Y17009)
B.terrae KMY02(AB201285)
B.phymatum STM815(AJ302312)
B.sabiae Br3407(AY773186)
B.phytofirmans PsJN(AY497470)
B.megapolitana A3(AM489502)
B.ginsengisoli KMY03(AB201286)
B.bryophila LMG23644(AM48950)
B.xenovorans LB400(U86373)
B.terricola LMG20594 (AY040362)
B.caledonica LMG 19076(AF215704)
B.fungorum LMG 16225(AF215705)
B.phenoliruptrix AC1100(AY435213)
B.graminis C4D1M(U96939)
Plant-associated beneficial and environmental group (PBE)
P. norimbergensis LMG13019(AF130171)
0.005

Figure 8. Phylogenetic tree of the *Burkholderia* genus based on 16S rRNA sequences (Suárez-Moreno *et al.*, 2012).

2.1.2 *Bukholderia glumae*

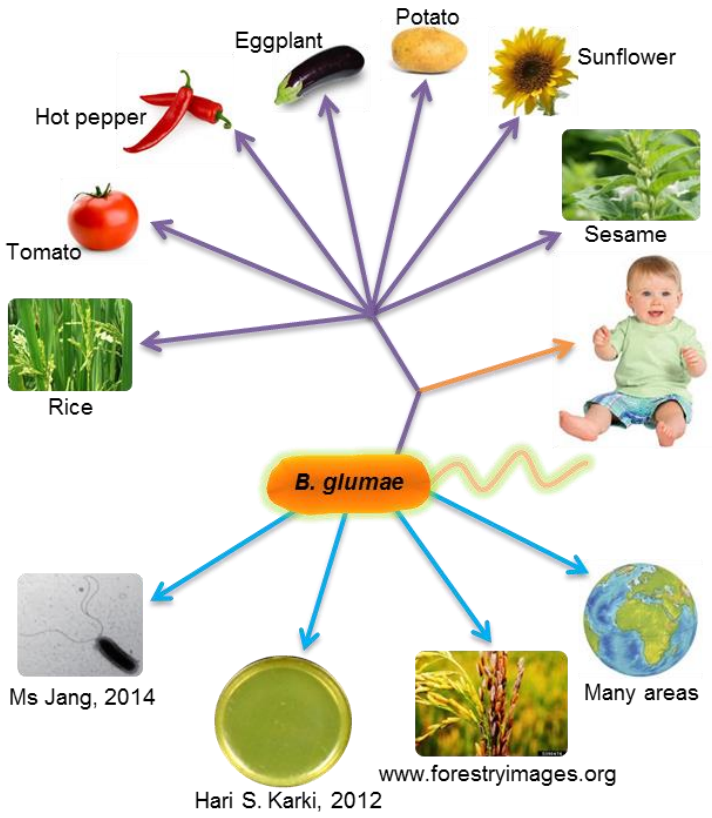


Figure 9. Lifestyle of *Burkholderia glumae*.

B. glumae was first isolated as a grain-rotting bacterium of rice in Japan in 1956, which can cause grain rot, sheath rot and seedling rot (Goto, K., Ohata, 1956). Since then *B. glumae* has been isolated from many rice-growing areas: Vietnam, Japan, China, Philippines, India, Africa and the USA (Chien, C. C.; Chang, 1987; Cottyn *et al.*, 1996; Jeong *et al.*, 2003; Luo *et al.*, 2007; Nandakumar *et al.*, 2007; Trung H.M., Van N.V., Vien N.V., 1993; Zeigler & Alvarez, 1989; Zhou, 2013). *B. glumae* is an aerobic, non-fluorescent, non-sporulating, rod shaped, Gram-negative bacterium, which could achieves motility by polar flagella. It can live at 11-50℃, and the optimum growth temperature is 30-35℃ (Ham *et al.*, 2011). Besides rice, *B. glumae* was reported to be capable of infecting many kinds of crops, including tomato, hot pepper, eggplant, potato, sunflower and sesame (Jeong *et al.*, 2003). Although it is not defined as a human pathogenic bacterium, a single case was reported that one strain of *B. glumae* was

isolated from the surgical specimens of a 8-month-old baby being diagnosed with the chronic granulomatous disease (CGD) (Weinberg *et al.*, 2007), indicating at least one strain of this pathogen could be associated with the opportunistic infections in immuno-compromised people (Figure 9).

2.2. QS in the genus *Burkholderia*

In the genus *Burkholderia,* two different QS systems have been identified, including the well-studied AHL-based QS system and the newly recognized DSF-based QS system (Deng *et al.*, 2011; Lewenza *et al.*, 1999). All *Burkholderia* species which have been investigated so far employ AHL signal molecules for cell to cell communication. Although the DSF synthase encoding genes are conserved in the genus *Burkholderia*, DSF was only characterized from few species of BCC group, for example, *B. cenocepacia, B. multivorans and B. vietnamiensis* (Deng *et al.*, 2010, 2011).

2.2.1. AHL-based QS in *Burkholderia*

The genus *Burkholderia* employs AHL-based QS systems to control pathogenicity, beneficial interactions of plant promotion, and the ability to degrade aromatic compounds. AHL-dependent QS systems identified in *Burkholderia* species and AHL molecules synthesized by these systems are presented in Table 2.

First evidence for the presence of AHL-mediated QS system in the *Burkholderia* genus was obtained in 1995 from *Burkholderia cepacia* by cross-feeding experiments with *Pseudomonas aeruginosa* (McKenney *et al.*, 1995). Later this AHL-based QS system was characterized from a clinical isolate of *B. cenocepacia* K56-2 by Lewenza et al and named as *cepIR*, which could facilitate the synthesis of C8-AHL and C6-AHL (Lewenza *et al.*, 1999). Further studies indicated that the *cepIR* system is conserved in the BCC group, which is composed of at least 17 species that are highly homologous in their 16S rRNA and *recA* sequences (Lutter *et al.*, 2001; Vanlaere *et al.*, 2009). Regulations of the *cepIR* system in BCC group mainly involve in the production of virulence factors, including protease, polygalacturonase, swarming motility, biofilm and siderophore ornibactine (Eberl, 2006). *Bptm* group, consisting of pathogens *B.*

pseudomallei, *B. mallei* and the non-pathogen *B. thailandensis*, has much more complicated QS systems than in other *Burkholderia* species (Majerczyk *et al.*, 2014a, b). *B. thailandensis* and *B. pseudomallei* possess three highly conserved LuxIR homologs, two of which *B. mallei* contains. In addition, each member of the *Bptm* group has two orphan LuxR homologs. Due to its non-pathogenicity, *B. thailandensis* is regarded as a promising bacterial model to investigate advantages and mechanisms of multiple QS systems.

Table 2. AHLs produced by the genus *Burkholderia*.

Bacterium	LuxR/I	AHL	Reference
B. cenocepacia	CepIR CciIR	C6-,C8-AHL	(Lewenza *et al.*, 1999) (Malott *et al.*, 2005)
B. multivorans	BmuIR	C8-AHL	(Yao *et al.*, 2002)
B. ambifaria	BafIR	C6-,C8-AHL	(Zhou *et al.*, 2003)
B. glumae	TofIR	C6-,C8-AHL	(Kim et al. 2004)
B. plantarii	PlaIR	C6-,C8-AHL	(Solis *et al.*, 2006)
B. vietnamiensis	CepIR BviIR	C6-,C8-,C10- AHL OC10-,C12-AHL	(Malott & Sokol, 2007)
B. mallei	BmaIR1 BmaIR3	C8-AHL OHC8-AHL	(Duerkop *et al.*, 2007) (Duerkop *et al.*, 2008)
B. unamae	BraIR	OHC10-,OC12-AHL	(Suárez-Moreno *et al.*, 2010) (Suárez-Moreno *et al.*, 2012)
B. pseudomallei	BpsIR1 BpsIR2 BpsIR3	C8-AHL OC8-AHL OHC10-AHL	(Gamage *et al.*, 2011)
B. phytofirmans	XenIR2 BraIR	OHC8-AHL OC14-AHL	(Coutinho et al., 2013)
B. xenovorans	XenIR2 BraIR	OC6-,OC8-,OC10-,OC12-AHL OHC10-,OC12-,OC14-AHL	(Coutinho *et al.*, 2013)
B. thailandensis	BtaIR1 BtaIR2 BtaIR3	C8-AHL OHC10-AHL OHC8-AHL	(Majerczyk *et al.*, 2014a)
B. glumae PG1	BgaIR1 BgaIR2 BgaIR2	Uncharacterized	(Knapp *et al.*, 2015) (Gao *et al.*, 2015)

2.2.2 QS in *B. glumae*

QS-system in *B. glumae* was first described in 2004, which is based on AHL. The AHL synthase and its regulator genes were named as *tofI* and *tofR* respectively in *B. glumae* BGR1 because the first identified role of this system is to regulate the production and transportation of toxoflavin, one of the key virulence factors in *B. glumae* (Kim *et al.*, 2004; Shingu & Yoneyama, 2004; Suzuki *et al.*, 2004). The *tofI* gene encodes for a 22.4 kDa LuxI homolog protein and the *tofR* gene encodes for a 26.6 kDa LuxR homolog protein (Jeong *et al.*, 2003; Kim *et al.*, 2004). Studies of thin layer chromatography (TLC) bioassay revealed that *B. glumae* BGR1 could produce two different AHL molecules: N-hexanoyl homoserine lactone (C6-AHL) and N-octanoyl homoserine lactone (C8-AHL). The mechanism of C6-AHL is not clear so far. C8-AHL has been demonstrated to combine with TofR to initiate transcription of *toxABCDE* and *toxFGHI* operons and promote production of toxoflavin by activating the toxoflavin production regulator ToxJ (Devescovi *et al.*, 2007; Kim *et al.*, 2004). In addition, toxoflavin combines with another toxoflavin regulator ToxR to induce transcriptional stimulation of *toxABCDE* and *toxFGHI* operons which in the end forms a positive feedback loop (Kim et al. 2004, 2007) (Figure 10).

Devescori's group found in *B. glumae* AU6028 that the *tofIR* QS system modulates the production and activity of lipase, which is another important virulence factor causing major symptoms of bacterial panicle blight in rice (Devescovi *et al.*, 2007) (Figure 10). Meanwhile, Giuliano and colleagues found that one of polygalacturonases, PehB, is under the control of *tofIR* QS system in strain AU6208 (Degrassi *et al.*, 2008) (Figure 10). As it is known that polygalacturonase is employed to degrade plant cell wall by various plant-associated bacteria, implicating polygalacturonase B as well as *tofIR* QS system has a role on the plant infection process of *B. glumae*.

Further studies indicated that C8-AHL mediated QS system played more roles in life style of *B. glumae*, such as motility. Researches performed by Kim.et.al revealed that QsmR, an IclR-type transcriptional regulator, regulates expression of the flagellum master transcriptional regulator FlhDC in *B. glumae* BGR1 (Figure 10). Since the QsmR is regulated by *tofIR* QS

system, suggesting *tofIR* QS system involved in flagellum biosynthesis and bacterial motility (Kim *et al.*, 2007). Moreover, Chun and colleagues found QsmR directly controls expression level of *katG* gene, which encodes an important catalase with a role in protecting bacterial cells from visible light (Chun *et al.*, 2009) (Figure 10). QsmR together with *tofIR* QS system also plays a role in modulating the expression of universal stress proteins in the strain BGR1 (Kim *et al.*, 2012) (Figure 10).

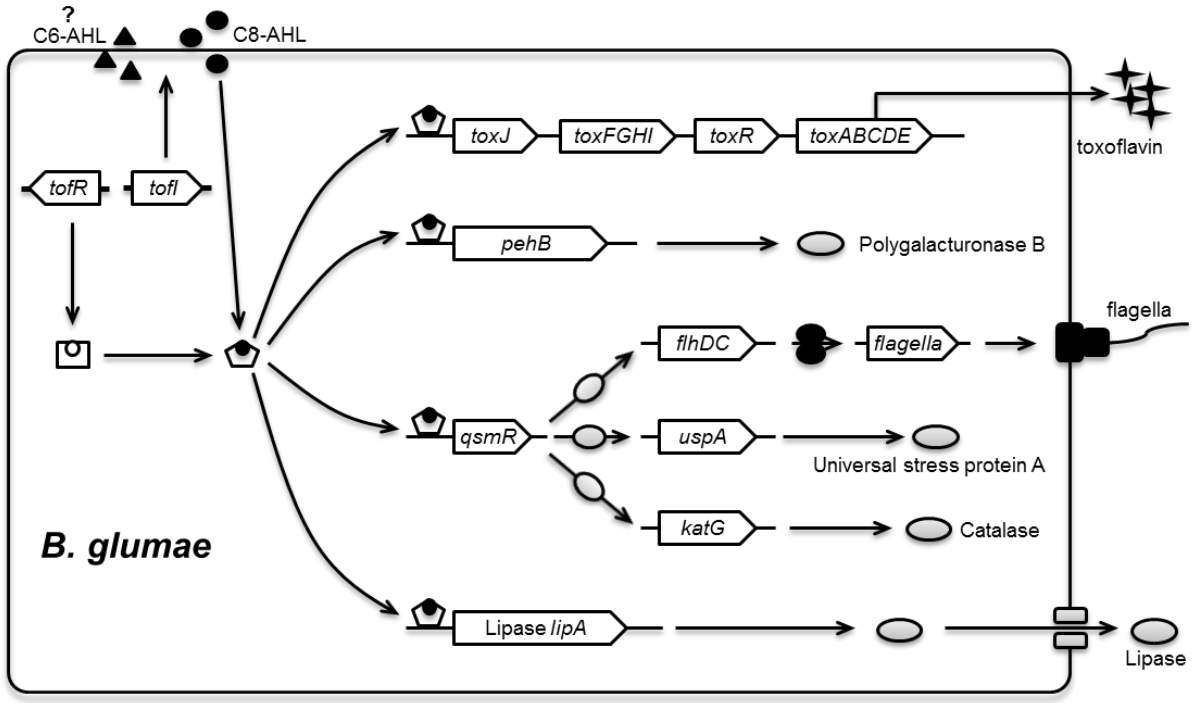


Figure 10. Phenotypes known to be regulated by AHL-mediated QS in *B. glumae*. C8-AHL mediated QS system regulates the synthesis and transport of toxoflavin, the expression of some enzymes, such as catalase and polygalcturonase, the production and activity of lipase, and controls bacterial motility by regulation expression of flagella genes.

3. Intentions of this research

Burkholderia glumae PG1 is a moderately pathogenic strain that is of great industrial interest. During the genome analysis of *B. glumae* PG1 three putative N-acyl-homoserine-lactone synthase genes were characterized in this study. The occurrence of three AHL synthases is surprising and in contrast to other *B. glumae* isolates in which only one AHL synthase gene was identified. However, it is a common feature within the genus *Burkholderia* to have multiple AHL synthase genes, whereas the presence of three AHL synthase genes is only unique in BGPG1 within the species *B. glumae* currently.

Within this study, the initial purpose was to study the function and regulatory circuits controlled by the three AHL-based QS systems in BGPG1. Therefore, the single deletion mutants had to be firstly constructed, and then phenotypes of these mutations were analyzed. Further RNA-seq analysis and qRT-PCR were employed to describe the complex regulatory network employed in this bacterium.

II. Material and Methods

1. Bacterial strains, constructs, vectors and primers

The bacterial strains, vectors, constructs and primers used in this study are listed in Table 3-5.

Table 3. Bacteria strains used in this study.

Bacterial strains	Description	Source/Reference
E. coli DH5α	*sup*E44,Δ*lac*U169 (Φ80*lacZ*ΔM15) *hsd*R17 *rec*A1 endA1 *gyr*A96 *thi*-1 *rel*A1	Gibco BRL, Eggenstein, Germany
E.coli WM3064	*thrB1*004 *pro thi rpsL hsdS lacZ*ΔM15 RP4–1360 Δ(*araBAD*)*567*Δ*dapA*1341::[*erm pir*(wt)]	(Dehio & Meyer, 1997)
B. glumae PG1	Wild type	(Frenken *et al.*, 1992)
B. glumae PG2	Δ*bgaI1* mutant of *bgaI1*, Gm^R	This study
B. glumae PG3	Δ*bgaI2* mutant of *bgaI2*, Gm^R	This study
B. glumae PG4	Δ*bgaI3* mutant of *bgaI3*, Gm^R	This study
B. glumae PG2c	pBBR1MCS-2 carrying the *bgaI1* gene	This study
B. glumae PG3c	pBBR1MCS-2 carrying the *bgaI2* gene	This study
B. glumae PG4c	pBBR1MCS-2 carrying the *bgaI3* gene	This study
B. glumae PG2p	BGPG2 harboring pBBRMCS-2	This study
B. glumae PG3p	BGPG3 harboring pBBRMCS-2	This study
B. glumae PG4p	BGPG4 harboring pBBRMCS-2	This study
A.tumefaciens NTL4	Reporter for AHL detection, *tra*I::*lac*Z, Tet^R, Sp^R	(Luo *et al.*, 2001)
C. violaceum CV026	Mini-Tn5 mutant of *C. violaceum* ATCC31532	(Mcclean *et al.*, 1997)

Table 4. Vectors and constructs used in this study.

Vectors/Constructs	Description	Source/Reference
pDrive cloning vector	Vector for PCR cloning, Amp^R and Km^R	QIAGEN
pDrive::*bgaI1*	pDrive vector with *bgaI1* from BGPG1	This study
pDrive::*bgaI2*	pDrive vector with *bgaI2* from BGPG1	This study
pDrive::*bgaI3*	pDrive vector with *bgaI3* from BGPG1	This study
pGEM-T vector	Vector for PCR cloning, Amp^R	Promega
pGEM-T::*bgaI1*U	668 bp clone of upstream of *bgaI1*	This study
pGEM-T::*bgaI1*D	762 bp clone of downstream of *bgaI1*	This study
pGEM-TΔ*bgaI1*	*bgaI1* deletion fragment in pGEM-T	This study
pGEM-TΔ*bgaI1*-Gm	deletion cassette Δ*bgaI1*-Gm in pGEM-T	This study
pGEM-T:: *bgaI2*U	754 bp clone of upstream of *bgaI2*	This study
pGEM-T:: *bgaI2*D	403 bp clone of upstream of *bgaI2*	This study
pGEM-TΔ*bgaI2*	*bgaI2* deletion fragment in pGEM-T	This study
pGEM-TΔ*bgaI2*-Gm	deletion cassette Δ*bgaI2*-Gm in pGEM-T	This study
pGEM-T:: *bgaI3*U	754 bp clone of upstream of *bgaI3*	This study
pGEM-T:: *bgaI3*D	403 bp clone of upstream of *bgaI3*	This study
pGEM-TΔ*bgaI3*	*bgaI3* deletion fragment in pGEM-T	This study
pGEM-TΔ*bgaI3*-Gm	deletion cassette Δ*bgaI3*-Gm in pGEM-T	This study
pNPTS138-R6KT	suicide plasmid; *MobRP4*$^+$ *ori*-R6K *sacB*; Km^R	(Lassak *et al.*, 2010)
pNPTS138-R6KT-Δ*bgaI1*-Gm	cassette of Δ*bgaI1*-Gm in pNPTS138-R6KT	This study
pNPTS138-R6KT-Δ*bgaI2*-Gm	cassette of Δ*bgaI2*-Gm in pNPTS138-R6KT	This study
pNPTS138-R6KT-Δ*bgaI3*-Gm	cassette of Δ*bgaI3*-Gm in pNPTS138-R6KT	This study
pBBR-MCS2	broad host-range vector, Km^R	(Kovach *et al.*, 1995)
pBBRMCS-2::*bgaI1*	pBBRMCS-2 with *bgaI1* gene	This study
pBBRMCS-2::*bgaI2*	pBBRMCS-2 with *bgaI2* gene	This study
pBBRMCS-2::*bgaI3*	pBBRMCS-2 with *bgaI3* gene	This study

Table 5. Primers used in this study.

Primer	Sequence[1)]	Source
M13-20 for	GTAAAACGACGGCCAGT	This study
M13 rev	CAGGAAACAGCTATGACC	This study
*bgal1*_f	ACGACATCGAGTTCGGCGTGTTC	This study
*bgal1*_r	AGCAGACCGTGTCTTCGGCATTG	This study
*bgal2*_f	GAGGCGGCGCGATACTATCAAC	This study
*bgal2*_r	CGCGAGATCGACGTGCTCAAGTG	This study
*bgal3*_f	AAAGATTGGGCACGCGATCGAATCC	This study
*bgal3*_r	ATCTTCAGCTTCCGCAGCTACCG	This study
*bgal1*_uf	CGGATCCGCGGACTATCCGGTTGCGATCCAC	This study
*bgal1*_ur	CAAGCTTGATCGACATCGACGCGCAGAC	This study
*bgal1*_df	CAAGCTTGCGGGAACACTTCCTGCAACAGGTAG	This study
*bgal1*_dr	GACGCGTCGTCGGCTGGGACTGGTATCTCGAAC	This study
*bgal2*_uf	GGGATCCGAGCTGCTCGAGGAATAC	This study
*bgal2*_ur	AGCAAGCTTCCAGTTTCTCGACGAACAC	This study
*bgal2*_df	ACTAAGCTTGCTTCAGCGCAGCAAAC	This study
*bgal2*_dr	GGAATTCGGGATCGTCGAGGGATG	This study
*bgal3*_uf	TGGATCCGTCATCGCTTGATGCTTGG	This study
*bgal3*_ur	CGAAAGCTTCAGGTGCTTGACGAAC	This study
*bgal3*_df	ACAAAGCTTACCGGAAGAAGGGATTCAG	This study
*bgal3*_dr	AGAATTCAGACCGCCGAGAACATCGTG	This study
*bgal1*_out_f	CGTGACGAACATGAGCGAACCCATC	This study
*bgal1*_out_r	ACAGCTCCCACGCTGTCATTCTTGC	This study
*bgal2*_out_f	AGGCGGACTTCTTCGGCTACCAG	This study
*bgal2*_out_r	CAGACCGTGATGATCTCGAACTACC	This study
*bgal3*_out_f	GCTTGTTCGCAGTGTAGTCCGAAGC	This study
*bgal3*_out_r	GTCGCGCTGATCTCGACGATCAACG	This study

[1)]: Primers were synthesized by Eurofins MWG Operon. Restriction sites are underlined.

2. Culture media, supplements and solutions

2.1. Culture media

All media and heat stable supplements used in this study were autoclaved at 121℃ for 20 min before use. Antibiotics and other heat sensitive supplements were sterile filtered (Rotilabo®-Spritzenfiler (CME, sterile, 0.22 µm), Roth, Germany) and added to media until they cooling down under 60℃.

2.1.1. Lysogeny broth (LB) medium

LB medium

NaCl	10g
Tryptone	5g
Yeast extracts	5g
Agar	15g
H_2O_{bidest}	ad 1000 ml

2.1.2. AT medium

AT salt solution (20x), AT buffer (20x) and Glucose solution (50%) were first prepared. To prepare 1 liter of 1 x AT working medium, 50 ml AT buffer (20x), 50 ml AT salt solution (20x), 10 ml glucose solution (50%) and 890 ml H_2O_{bidest} were combined.

AT salt solution (20x)

$(NH_4)_2SO_4$	40g
$MgSO_4$ x 7 H_2O	3.2g
$CaCl_2$ x 2 H_2O	0.2g
$FeSO_4$ x 7 H_2O	0.1g
$MnSO_4$ x 7 H_2O	0.024g
H_2O_{bidest}	ad 1000 ml

AT buffer (20x)

KH_2PO_4	214g
H_2O_{bidest}	ad 1000 ml
	Adjust to pH 7.0 and autoclave

Glucose solution (50%)

Glucose	50g
$H2O_{bidest}$	ad 100 ml
	Sterile filtered

2.2. Supplements

Table 6. Supplements

Supplements	Abbreviations	Solvents	Stock concentrations	Working concentrations
Ampicillin	Am	H_2O_{bidest}	100mg/ml	100µg/ml
Chloramphenicol	Cm	EtOH	25mg/ml	25µg/ml
Gentamycin	Gm	H_2O_{bidest}	50mg/ml	50µg/ml
Kanamycin	Km	H_2O_{bidest}	25mg/ml	25µg/ml
Spectionomycin	Sp	H_2O_{bidest}	50mg/ml	50µg/ml
Tetracyclin	Tc	H_2O_{bidest}	5mg/ml	5µg/ml
Congo Red		H_2O_{bidest}	5mg/ml	50µg/ml
X-Gal*		DMF	50mg/ml	50µg/ml
IPTG		H_2O_{bidest}	100mg/ml	100µg/ml
DAP		H_2O_{bidest}	30mM	300µM
Tributyrin	TBT	Medium	-	1%(v/v)

*X-Gal: 5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside

3. Cultivation of bacteria

Bacteria were inoculated in liquid medium or on solid medium using either single colonies from LB agar plates or aliquots of culture medium from related strain collection. Inoculation was carried out with a sterile pipette tip or an inoculation loop. In liquid medium, bacteria were grown in Erlenmeyer flasks, test tubes at 150-250 rpm in rotary shakers (Infors HT, Mintron, Bottmingen, Switzerland) at appropriate temperature. In solid medium, they were cultured with agar in petri dishes in incubator at appropriate temperature. Bacterial strains harboring specific plasmids were supplemented with related antibiotics in order to keep them under selection pressure.

3.1. Cultivation of *B.glumae* strains

B. glumae was cultured 1-2 days at 30℃ in LB medium supplemented with chloramphenicol (25 µg/ml) and other appropriate antibiotics.

3.2. Cultivation of *Escherichia coli* strains

E. coli strains were grown overnight at 37℃ in LB medium supplemented with appropriate antibiotics and supplements (Table 6).

3.3. Cultivation of *Agrobacterium tumefaciens* NTL4

A. tumefaciens NTL4 (here called NTL4) from glycerol stock was inoculated in 5 ml of LB medium supplemented with spectionomycin (50µg/ml) and tetracycline (5µg/ml) and cultured overnight at 30℃. The following day 100 µl of the preculture was inoculated in 5 ml of AT medium supplemented with spectionomycin and tetracycline and incubated overnight at 30℃.

3.4. Cultivation of *Chromobacteium violaceum* CV026

C. violaceum CV026 (here called CV026) from glycerol stock was inoculated in 5 ml of LB medium supplemented with chloramphenicol (25 µg/ml) and cultured overnight at 30℃.

3.5. Maintenance of bacterial strains

Bacterial colonies growing on agar plates were stored for up to 1 month at 4℃. For long term storage, 0.6 ml overnight bacteria culture was mixed with 0.3 ml glycerol (66%) in a screw-cap tube and stored at -80℃.

3.6. Quantification of bacterial growth

Bacterial growth was determined by measuring optical density in a 1-cm path length cuvette (Sarstedt, Nuembrecht, Germany) at the wavelength of 600 nm (OD_{600}) with a photometer (Eppendorf AG, Hamburg, Germany). The sterile medium was used as reference. The OD_{600} value of 0.1 corresponds to a cell density of approximately $1x10^8$cells/ml for *E.coli*.

4. Standard techniques for working with DNA

All the materials and solutions were first sterilized either by autoclaving at 121℃ for 20 min or by sterile filters with a pore size of 0.22 µm.

4.1. Isolation of plasmid DNA

Isolation of plasmid DNA was performed with the High-Speed Plasmid Mini Kit (Geneaid Biotech Ltd., Taiwan, China). Cells from overnight culture (3 ml) were harvested by centrifugation at 13,000 rpm for 1 min. The pellet was suspended in 200 µl of PD1 Buffer supplemented with RNase A by vortex. Then 200 µl of PD2 Buffer was added and mixed gently by inverting the tube 10 times. Keeping the tube at room temperature for 2-5 minutes, 300 µl of PD3 was added and centrifuged at 13,000 rpm for 3 min. The supernatant was transferred into a new PD column located in a Collection Tube and centrifuged for 30 sec. The flow-through and Collection Tube were discarded and the column was placed in a new collection tube and washed with 400 µl W1 Buffer and 600 µl Wash Buffer, each followed by a centrifugation step of 30 sec. To completely remove residual buffer, the column was centrifuged again for 2 min. The DNA was eluted by adding 50 µl sterile H_2O_{bidest}. Then the column was centrifuged at 13,000 rpm after 2 min of incubation at room temperature. The plasmid DNA was stored at -20℃.

4.2. Isolation of genomic DNA

Genomic DNA from BGPG1 was isolated by using the Aqua Pure Genomic DNA Kit (Bio-Rad Laboratories, Hercules, Canada). Cells from an overnight culture (less than 1 x 10^9 cells) were harvested by centrifugation at 4,000 g for 10 min at room temperature. Cell pellet was resuspended in 190 µl TE Buffer, and then 10 µl lysozyme solution (10 mg/ml) was added. The tube was incubated at 30℃ in a water bath with shaking for 10 min and followed by centrifugation at 4,000 g for 5 min at room temperature. After that, the pellet was resuspended in 400 µl DNA Lysis Buffer T, then 20 µl Proteinase K solution and 15 µl RNase A (20 mg/ml) were added. The mixture was incubated in a water bath at 70℃ with shaking for 30 min for an efficient lysis. After that 200 µl DNA Binding Buffer was added and mixed by pipetting, the entire lysate including precipitate was transferred into a Perfect Bind DNA Column located in a Collection Tube and centrifuged for 1 min at 10,000 g for DNA binging. The flow-through and Collection Tube were discarded. The column was washed twice using 650 µl of DNA Wash Buffer, each followed by a centrifugation step of 1 min. To dry the column matrix, the column

was centrifuged again at 10,000 g for 2 min. 50-100 µl of the sterile H_2O_{bidest} was added to the column to elute the genomic DNA. The genomic DNA was stored at -20℃ or -80℃.

4.3. Purification and concentration of DNA

DNA product from PCR was purified by the Gel/PCR DNA Fragments Extraction kit (Avegene life science, China), following the PCR cleanup protocol. DNA was eluted with 20 to 50 µl sterile H_2O_{bidest}. The concentration of DNA solution was performed with a vacuum concentrator (Concentrator 5301, Eppendorf, Hamburg, Germany) at 45℃ for up to 5 min.

4.4. Determination of DNA concentration

The Eppendorf BioPhotometer (Eppendorf AG, Hamburg, Germany) was used to determine concentration of DNA in a disposable micro UV cuvette (Plastibrand®, Brand, Wertheim, Germany) at 260 nm against sterile H_2O_{bidest}. The concentration of pure double-stranded DNA with an OD_{260} of 1.0 is normalized as 50 mg/ml. The purity of DNA is measurable by the ratio of OD_{260}/OD_{280}, which usually is in the range of 1.8 to 2.0 (Sambrook & Russell, 2001).

4.5. Agarose gel electrophoresis

50x TAE buffer

EDTA	100 mM
Tris	2 M
H_2O_{bidest}	ad 1000 ml
pH 8.1 (adjusted with acetic acid)	

Loading dye

Glycerol (30%)	60 ml
EDTA	50 mM
Bromophenol blue (0.25%)	0.5 g
Xylencyanol (0.25%)	0.5 g
H_2O_{bidest}	ad 200 ml

DNA fragments were separated by 0.8-2 % agarose gels in 1x TAE buffer. DNA samples were mixed with 1/10 volume of loading dye and loaded onto gels. Gels were run at 100 V for 20-60 min with a power supply (Amersham Biosciences, Piscataway, NJ, USA) in a gel chamber (HE-33 mini horizontal submarine unit, Hoefer™, Holliston, MA, USA) filled with 1x TAE buffer. After that, Gels were stained for 5-15 min in an ethidium bromide solution (10 µg/ml) and visualized under UV light at 254 nm by a Molecular Imager® (GelDoc TM XR+ Imaging System, BioRad, Munich, Germany). Pictures were analyzed by Quantity One 1-D analysis

software (version 4.6.9, BioRad, Munich, Germany). The size of DNA fragment was determined by comparison to the standard GeneRuler™ 1 kb DNA Ladder (Fermentas, St. Leon-Rot, Germany).

4.6. DNA extraction from agrose gel

Desired DNA fragments were excised from agarose gels by a razor blade under UV light and extracted by a Gel/PCR DNA Fragments Extraction kit (Avegene life science, Taiwan, China) according to the manufacturers' instructions. DNA fragments were eluted in two consecutive aliquots of 15 µl sterile H_2O_{bidest}. To determine the quality of extraction, 2-3 µl purified DNA was analyzed by agarose gel electrophoresis using the standard marker as a reference.

4.7. Enzymatic modification of DNA

4.7.1. Restriction analysis of DNA

Analytical digestion	
DNA solution	1- 2 µl
Reaction buffer (10x)	1 µl
Restriction enzyme	0.5 µl
Sterile H_2O_{bidest}	ad 10 µl

Preparative digestion	
DNA solution	5-10 µl
Reaction buffer (10x)	5 µl
Restriction enzyme	2 µl
Sterile H_2O_{bidest}	ad 50 µl

DNA restriction was done by digestion with specific enzymes. 1 unit of restriction enzyme was used to digest 1 µg of DNA at appropriate temperature according to recommendations of producer. Analytical digestions were incubated for 1-3 h, and preparative reactions were incubated overnight. Heat treatment is applied to inactivate restriction enzymes at 65℃ or 80℃ for 20 min after digestion. All restriction enzymes and related buffers were purchased from Fermentas (St. Leon-Rot, Germany).

4.7.2. Ligation of DNA fragments

4.7.2.1. Ligation of DNA fragments into the pDrive cloning vector

After purification, DNA fragments were cloned into the pDrive vector using the QIAGEN®PCR Cloning Kit (QIAGEN, Hilden, Germany) according to the manufacturer's instructions. After incubation at 16℃ for 1-2 h or overnight, an aliquot of the ligation mixture was directly used to

transform competent cells of *E. coli* DH5α by heat shock.

pDrive ligation

pDrive vector	0.5 µl
PCR product	0.5 - 2 µl
Ligation master mix	2.5 µl
Sterile H_2O_{bidest}	ad 5 µl

4.7.2.2. Ligation of DNA fragments into the pGEM-T cloning vector

DNA fragments were ligated into the pGEM-T vector (Promega, Mannheim, Germany) according to producer's recommendations. After incubation at 16℃ for 1-2 h or overnight, an aliquot of the ligation mixture was directly used to transform the competent cells of *E. coli* DH5α by heat shock.

pGEM-T ligation

pGEM-T vector	0.5 µl
DNA fragment	0.5 - 2 µl
Ligation master mix	2.5 µl
Sterile H_2O_{bidest}	ad 5 µl

4.7.2.3. Ligation of DNA fragments with T4 DNA ligase

Ligation

Vector	x µl
Insert DNA	y µl
Ligase buffer (10x)	2 µl
T4 ligase	0.5 µl (sticky ends)
	1 µl (blunt ends)
H_2O_{bidest}	ad 20 µl

DNA fragments generated by restriction digestion or PCR products amplified with *Pfu* polymerase (Fermentas, St. Leon-Rot, Germany) were combined with cloning vectors either by sticky ends or blunt ends. The molar ratio of vector to insert was 1:2. After incubation at 16℃ for 1-2 h or overnight, an aliquot of the ligation mixture was directly used to transform the competent cells of *E. coli* DH5α or *B. glumae* by heat shock.

4.7.3. Generation of blunt-end DNA fragments by *Pfu* ploymerase

To generate blunt ends, *Pfu* polymerase (Fermentas, St. Leon-Rot, Germany) was used to remove overhangs of DNA fragments produced by restriction enzymes or polymerases. The reaction was incubated at 37℃ for 1 h and followed by a heat step at 70℃ for 10 min to inactivate *Pfu* polymerase. DNA fragments were directly purified following procedures described in II.4.3 or extracted from agarose gels (II.4.6).

Blunt-ends reaction (20 µl)

DNA solution	17.5 µl
Pfu polymerase	0.5 µl
Buffer (10x)	2 µl

4.7.4. Generation of A-tailing DNA fragments by *Taq* polymerase

A-tailing (10 µl)

DNA solution	7.5 µl
dATP (2mM)	1.0 µl
Taq ploymerase	0.5 µl
Taq buffer (10x)	1.0 µl

Non-template-dependent A-tailings were added to 3´-blunt ends of DNA fragments with Taq polymerase, which allowed DNA fragments to be ligated into TA cloning vectors (e.g., pGEM-T vector). The reaction lasted for 30 min at 70℃.

4.8. Polymerase chain reaction (PCR)

Polymerase chain reaction (PCR) was employed to specifically amplify defined DNA fragments in a Master cycler personal (Eppendorf Hamburg, Germany).

4.8.1. Primers

PCR primers were designed using the “Clone Manager 9.0” program purchased from Scientific & Educational Software (Cary NC, USA) and listed in the Table 5. The annealing

temperature (T_{ann}) of a PCR depended on the melting temperature (T_m) of the primers. Generally, the T_{ann} was 5℃ lower than the lower T_m of the pair of primers used (Chester & Marshak, 1993).

4.8.2. Standard PCR reactions

Standard PCR was performed either with Taq polymerase or *Pfu* polymerase in 100 µl PCR tubes. The mixture of PCR was always prepared on ice to avoid false annealing. Volumes of the reaction components are listed in Table 7. The cycling conditions for standard PCR are listed in Table8.

Table 7. Standard PCR reaction (50 µl).

Components	Volume	Final concentration
DNA(template)	1 µl	50-1000 pg/µl
Polymerase	1 µl	0.5µM
buffer (10x)	5 µl	1x
dNTPs (10 mM)	1 µl	200 µM
Primer forward	5 µl	1 µM
Primer reverse	5 µl	1 µM
DMSO	4 µl	
Sterile H_2O_{bidest}	ad 50 µl	

Table 8. Standard PCR conditions.

PCR steps		Temperature	Time
Initial denaturation		95℃	5 min
Steps cycle (25-35)	Denaturation	95℃	40 sec
	Annealing	$T_{ann}=T_m$-5℃	50 sec
	Elongation	72℃	1 min/1 kb (*Taq*) or 1 min/0.5 kb (*Pfu*)
Final elongation		72℃	10 min

4.8.3. Direct colony PCR

Direct colony PCR was used to quickly identify and select plasmids containing correct inserts directly from bacterial colonies. Simply, single colony was picked and added to PCR reaction

mixture instead of DNA templates. Volumes of the reaction components are listed in Table 9. The cycling conditions for direct colony PCR are in Table 10.

Table 9. Direct colony PCR reaction (25 µl).

Components	Volume	Final concentration
colony	Picked	
Polymerase	0.5 µl	0.5µM
buffer (10x)	2.5µl	1x
dNTPs (10 mM)	0.5µl	200 µM
Primer forward	2.5 µl	1 µM
Primer reverse	2.5µl	1 µM
DMSO	2 µl	
Sterile H_2O_{bidest}	ad 25 µl	

Table 10. Direct colony PCR conditions.

PCR steps		Temperature	Time
Initial denaturation		95℃	10 min
Steps cycle (25-35)	Denaturation	95℃	40 sec
	Annealing	$T_{ann}=T_m-5$℃	50 sec
	Elongation	72℃	1 min/kb
Final elongation		72℃	10 min

4.9. DNA transfer techniques

4.9.1. DNA transformation of *E. coli* cells using heat shock

4.9.1.1. Preparation of competent *E. coli* cells

1ml fresh overnight culture of *E.coli* cells was inoculated in 100 ml LB medium supplemented with appropriate antibiotics and supplements and incubated at 37℃ to an OD_{600} of 0.5. Cells were placed in an ice bath for 10 min and harvested by centrifugation at 4,000 rpm for 10 min at 4℃. The cell pellet was resuspended in 20 ml ice-cold 0.1M $CaCl_2$ with 10% glycerol (sterile filtered) by swirling on ice gently followed by incubation on ice for additional 45 min. Cells were spun down and resuspend again as above. After a third centrifugation, cell pellet was resuspened in 2 ml ice-cold 0.1M $CaCl_2$ with 10% glycerol and aliquots were made by

pipetting 100 µl of resuspension into 1.5 ml sterile freezing microfuge tubes and stored at -80℃.

4.9.1.2. Heat shock transformation of *E. coli* cells

An aliquot of competent *E. coli* cells was thawed on ice and mixed with up to 10 µl ligation products by pipetting gently. After incubation on ice for 30 min, heat shock was performed at 42℃ for 90 sec. Cells were incubated on ice for another 5 min. Then 800 µl LB medium without antibiotics was added and the cells were incubated at 37℃ for 2 h with shaking. 100 µl culture was plated on a LB agar plate containing appropriate antibiotics and supplements. The plate was inverted and incubated overnight at 37℃.

4.9.2. Conjugation of the plasmid DNA into *B. glumae*

1 ml overnight LB culture of *E.coli* WM3064 containing appropriate plasmids as a donor and 1 ml overnight LB culture of *B. glumae* as a recipient were mixed by vortex and centrifuged at 13,000 rpm for 5 min. The cell pellet was washed twice by first resuspended in 1 ml LB medium and second resuspended in 500 µl LB medium. 50 µl of mixture was spotted on a LB agar plate supplemented with DAP (300 µM) followed by incubation at 30℃ overnight. The whole colony was taken by sterile pipette tip from the plate and transferred into 1 ml of LB medium. After spinning down, the pellet was washed twice using fresh LB medium. Then limiting dilution was done from 10^{-1} to 10^{-5}. Dilution samples were plated on LB plates containing appropriate antibiotics and incubated at 30℃ overnight. Afterwards white clones were picked up and verified by PCR.

4.10. Sequencing for vector constructs and PCR products

Sequencing was performed by the group of Prof. Schreiber in Institute for clinical Molecular biology (University Hospital of Schleswig-Holstein, Kiel, Germany) using an ABI 3730XL DNA Analyzer (Applied Biosystems, Carlsbad, CA, USA) based on the Sanger technique (Sanger & Nicklen, 1977).

DNA required to be sequenced were purified and adjusted to a concentration of 100 ng/µl

Appropriate primers were diluted to 4.8 µM in sterile H_2O_{bidest}. One sequencing sample contained 3 µl of DNA and 1 µl of primer.

4.11. Construction of the deletion mutants of *bgaI1-3*

4.11.1. DNA constructs for the deletion mutants of *bgaI1-3*

DNA constructs for deletion mutants of BGPG1 generated in this study are listed in Table 4. PCR primers used to create and confirm deletion mutants are listed in Table 5. All deletion mutants generated in this study were produced by double-crossover homologous recombination in the flanking regions of targeted genes with the suicide vector pNPTS138-R6KT. To delete *bgaI1* gene, the two flanking regions of *bgaI1* gene were PCR amplified with primers of *bgaI1*_uf/*bgaI1*_ur and *bgaI1*_df/*bgaI1*_dr. Then the two fragments were cloned into pGEM-T vector seperately to generate pGEM-T::*bgaI1U* and pGEM-T::*bgaI1D* by TA cloning. To connect the two fragments, pGEM-T::*bgaI1U* and pGEM-T::*bgaI1D* were both digested by enzyme HindIII and PciI. The large fragment (3.2kb) from pGEM-T::*bgaI1U* which contains the *bgaI1U* and the small fragment (1.2kb) from pGEM-T::*bgaI1D* which contains *bgaI1D* were purified from gel. The two purified fragments were ligated with T4 ligase to generate pGEM-T::*bgaI1U-bgaI1D*. Gm^R gene as the antibiotic resistance selection marker was amplified by *Pfu* polymerase with GmR_HindIII_f and GmR_HindIII_r as primers and vector pBBRMCS-5 as template. Gm^R gene was inserted into pGEM-T::*bgaI1U-bgaI1D* between *bgaI1U* and *bgaI1D* by digestion the plasmid with HindIII and ligated with T4 ligase to produce pGEM-T-Δ*bgaI1*-Gm. Then the Δ*bgaI1*-Gm cassette was PCR amplified by *Pfu* polymerase and inserted into the suicide vector pNPTS138-R6KT to obtain the pNPTS138-R6KT-Δ*bgaI1*-Gm. By using the same procedures the pNPTS138-R6KT-Δ*bgaI2*-Gm and pNPTS138-R6KT-Δ*bgaI3*-Gm were obtained.

4.11.2. Allelic exchange of the *B. glumae* genome for *bgaI1-3* deletions

The pNPTS138-R6KT- Δ*bgaI*-Gm cassettes were first introduced into *E. coli* WM3064 by heat shock and positive clones were selected by Gm. Then one positive clone was selected as a donor to do biparental conjugative mating with BGPG1 on LB medium only containing DAP (II

4.9.2). After overnight incubation, colonies were picked, washed, diluted and plated on LB agar plates containing Gm and Km but lacking DAP. Positive clones were picked and inoculated in LB broth and grown overnight at 30℃. The overnight culture was then plated onto LB agar plate containing 10% (w/v) sucrose but lacking kanamycin for plasmid excision by secondary homologous recombination. Single sucrose-resistant colony was confirmed by appropriate PCR. The primers are listed in the Table 5. The obtained mutations were verified by sequencing and designated as BGPG2 for BGPG1-Δ*bgaI1*, BGPG3 for BGPG1-Δ*bgaI2* and BGPG4 for BGPG1-Δ*bgaI3* (Table 3).

4.12. DNA constructs for complementation of *bgaI* mutants

To obtain three complementation constructs, pBBRMCS-2::*bgaI1*, pBBRMCS-2::*bgaI2* and pBBRMCS-2::*bgaI3*, *bgaI1-3* were PCR-amplified with *Pfu* polymerase using following primers *bgaI1*_f and *bgaI1*_r*, bgaI2*_f and *bgaI2*_r and *bgaI3*_f and *bgaI3*_r*,* respectively (Table 5). Then, PCR products were initially inserted into pBBRMCS-2 vector. After that, these 3 constructs were introduced into 3 mutant strains BGPG2-4, respectively by conjugation with the donor strain of *E. coli* WM 3064 (II 4.9.2).

5. AHL production assay

To detect AHL production, *A. tumefaciens* NTL4 was used as an AHL bioreporter (Luo *et al.*, 2001; Shaw *et al.*, 1997). *A. tumefaciens* NTL4 has no AHL synthase gene and cannot produce any compounds with detectable AHL activity. However, it can detect exogenous AHL activity because it contains a pZLR4 plasmid with a copy of *traR* and a fusion of *traG* and *lacZ* in this reporter. Exogenous AHL can bind to the transcription regulator TraR and activate the *traG*::*lacZ* fusion which promotes production of β-galactosidase. This released enzyme can convert a colorless substrate (X-Gal or ONPG) into a blue or yellow product (Fiugure 10).

The second AHL bioreporter is *C. violacein* CV026. Wild type *C. violaceum* produces a natural antibiotic called violacein, which is a purple and water-insoluble pigment. Its production is regulated by the C6-AHL in *C. Violaceum.* However, AHL reporter CV026 carries a mutation of the *cviI* gene and can't produce any detectable AHL molecules. The presence of exogenous

AHLs recovers its ability to produce purple pigment violacein (Mcclean *et al.*, 1997) (Figure 11).

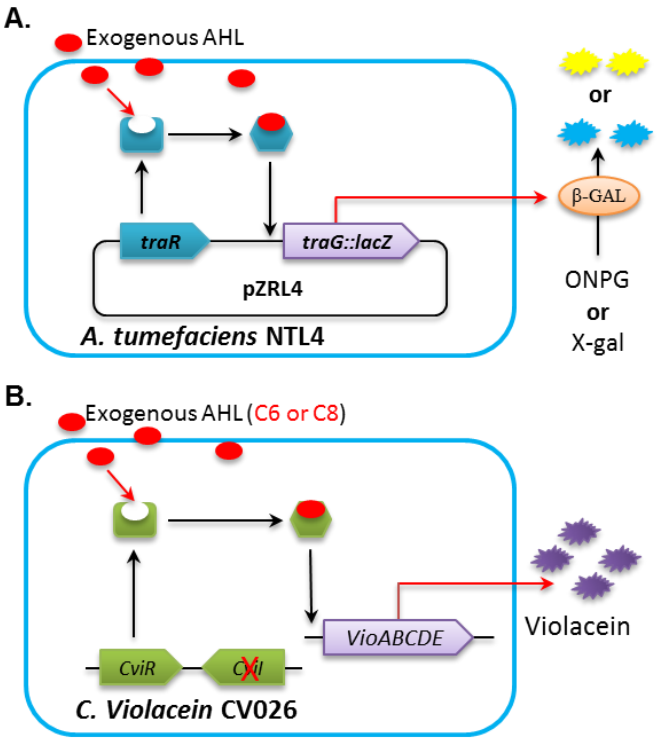


Figure 11. Detection of exogenous AHL molecules by AHL reporter strains. (A) *A. tumefaciens* NTL4. **(B)** *C. violaceum* CV026.

5.1. Extraction of AHL from cell-free supernatants

B. glumae strains were grown for 28 h and *E. coli* was grown for 24 h in 100 ml of medium to stationary phase. After centrifugation at 10,000 rpm for 25 min, the supernatant was mixed with 300 ml of ethyl acetate (0.1% v/v glacial acetic acid) and shook vigorously for 15 min. The organic layer was collected in a separation funnel and dried over anhydrous magnesium sulphate, filtered, and evaporated to dryness in a Rota vapor (Buchi RE 111, Switzerland). Residues were dissolved in 500 µl of ethyl acetate and stored at -20℃.

5.2. AT soft agar screening using *A. tumefaciens* NTL4 (Schipper, 2009)

The AT soft agar screening was performed to detect the AHL production in bacteria culture using the AHL reporter bacteria *A. tumefaciens* NTL4. Preparation of AT soft agar was given below.

AT soft agar (100 ml)

Eiken agar	1g
H_2O_{bidest}	89ml
	Autoclaved (121℃, 20 min)
AT buffer(20x)	5ml
AT salt solution(20x)	5ml
50% Glucose	1ml
Spectinomycine(50 mg/ml)	120 µl
X-Gal(50 mg/ml)	120 µl

A preculture of *A. tumefaciens* NTL4 was grown overnight in 5 ml AT medium with Sp and Tc (Table 6) at 30℃, and then added to the AT soft agar to a final cell density of 10^7 cells/ml. 25 ml of AT soft agar was poured into the petri dish and 20 µl of bacterial culture was pipetted onto the solidified agar. The plates were incubated at 30℃ overnight. 5 µl of 10^{-6} M C8-AHL in ethyl acetate was used as the positive control and and the empty pDrive vector used as negative control.

5.3. ONPG assay using *A. tumefaciens* NTL4

Z-buffer stock solution

Na_2HPO_4	4.27 g
$NaH_2PO_4 \times H_2O$	2.75 g
KCl	0.375 g
$MgSO_4 \times 7H_2O$	0.125 g
	Adjust to pH 7.0
Sterile H_2O_{bidest}	ad 500 ml
	Do not autoclave. Store at 4℃

Complete Z-buffer

Z-buffer	50 ml
β-mercaptoethanol	0.14 ml

ONPG (4 mg/ml)

ONPG	80 mg
Sterile H_2O_{bidest}	20 ml

Na_2CO_3 (1 M)

Na_2CO_3	5.3 g
Sterile H_2O_{bidest}	50 ml

The ONPG assay was performed to quantify AHL production in the culture of bacteria using *A. tumefaciens* NTL4. This reporter was grown overnight in 5 ml AT medium with Sp and Tc (Table6) at 30℃ and diluted in 5ml AT medium to a cell concentration of 1×10^7cells/ml. Then, 5 µl of the AHL ethyl acetate extracts from bacteria culture supernatant was added. After overnight incubation at 30℃, the cell density was determined at OD_{600}. 1 ml of cell suspension

was vortexed with 20 µl toluene for 5 min. Then 800 µl solution of the lower layer was transferred into a new 1.5 ml Eppendorf tube and mixed with 200 µl of freshly prepared ONPG-solution (4 mg/ml) in Z-buffer. After 20 min incubation at room temperature, 400 µl of 1M Na_2CO_3 solution was added and this mixture was centrifuged at 13,000 rpm for 2 min. Optical density of the supernatant of mixture was measured at 420 nm by a SmartSpec™ Plus Spectrometer (Bio-Rad Laboratories, Munich, Germany) and the OD_{420}/OD_{600} was calculated. 5 µl of 10^{-6} M C8-AHL in ethyl acetate was used as the positive control and and the empty pDrive vector used as negative control.

5.4. Violacein production assay

To screen AHL sythase gene, AHL production was detected in LB agar plate by bioreporter assay with *C. violaceum* CV026 (Ravn *et al.*, 2001). Briefly, 5 µl cultures of clones and reporters were inoculated in parallel on LB agar plates and incubated at 30℃ overnight.

For the liquid medium assay, violacein was extracted by a previously described method with a slight modification (Blosser & Gray, 2000). CV026 was grown overnight in LB medium and diluted in sterile LB medium to an optical density (600 nm) of 0.002. 5 µl AHL extract was added and CV026 was continually incubated at 30℃ for 16 h with shaking. After vortexing, 200 µl of culture was placed in a new Eppendorf tube. 200 µl of 10% sodium dodecyl sulfate (SDS) was added to lyse cells, followed by vortexing for 10 sec and incubating at room temperature for 5 min. 900 µl of water-saturated butanol was added to extract violacein, followed by vortexing for 10 sec and centrifuging for 5 min at 13,000 rpm. The upper phase containing the violacein was harvested and its OD_{585} was measured in a SmartSpec™ Plus Spectrometer (Bio-Rad Laboratories, Munich, Germany). The relative violacein amount was calculated as the ratio of OD_{585}/OD_{600}. 5 µl of 10^{-6} M C8-AHL in ethyl acetate was used as the positive control and and the empty pDrive vector used as negative control.

5.5. Analysis of AHLs using thin layer chromatography (TLC) overlay assay

To further differentiate the AHL molecules by the variety of acyl chain, TLC overlay assay was

carried out using the *A. tumefaciens* NTL4 as a AHL reporter (Shaw *et al.*, 1997). 2 µl of each cell-free culture extract was spotted individually onto TLC Silica gel 60 RP-18 F_{254S} plate (Merck KGaA, Darmstadt, Germany). Spots were dried in the fuming cupboard. AHL molecules were separated using the methanol/water (60:40 v/v) mobile phase until the solvent line was approximately 1.5 cm from the top of the TLC plate. After that, TLC plates were dried and then overlaid with 1% AT soft screening agar containing *A. tumefaciens* NTL4 (10^7 cells/ml) (Zhu *et al.*, 1998). Overlaid TLC plates were incubated overnight at 30℃ and pictures were taken.

6. Motility assay

6.1. Swarming and Swimming assay

Swarming and swimming assays were performed using swarming and swimming agars in petri dish as previously described with modifications (Bijtenhoorn *et al.*, 2011). Following solutions were first prepared, sterile filtered and stored at room temperature.

Solution 1	
Glucose	50g
H_2O_{bidest}	ad 100 ml

Solution 2	
$MgSO_4$ x 7 H_2O	2g
H_2O_{bidest}	ad 100 ml

Solution 3	
$CaCl_2$ x 2 H_2O	0.2g
H_2O_{bidest}	ad 100 ml

Solution 4	
$C_5H_8NNaO_4$ x H_2O	5.5g
H_2O_{bidest}	ad 100 ml

Solution 5a	
Na_2HPO_4	7g
KH_2PO_4	3g
NaCl	0.5g
NH_4Cl	1g
H_2O_{bidest}	ad 100 ml

Solution 5b	
Na_2HPO_4	7g
KH_2PO_4	3g
NaCl	0.5g
H_2O_{bidest}	ad 100 ml

Swarming or swimming agar was prepared as described in Table 11 and poured into petri dishes. Overnight cultures of *B. glumae* strains were centrifuged and washed twice by fresh LB medium. 5 µl of fresh LB medium containing 1×10^7 cells was spotted in the middle of the agar plate and incubated at 30℃ (Kim *et al.*, 2007). After 3 days incubation, the plates were

documented by photography.

Table 11. Preparation of swarming or swimming agar (100 ml).

Solutions	Swarming agar (ml)	Swimming agar (ml)
Solution1	2	2
Solution2	1	1
Solution3	1	1
Solution4a	10	-
Solution4b	-	10
Solution5	1	-
Eiken agar	0.45 g in 85 ml H_2O_{bidest} Atutoclaved (121℃, 20min)	0.25 g in 86 ml H_2O_{bidest} Atutoclaved (121℃, 20min)

6.2. Sedimentation assay

Sedimentation assays were performed as described previously (Krysciak *et al.*, 2014) to identify BGPG1 motility in liquid. Firstly, the precultures of *B. glumae* strains were established in 5 ml LB medium and cultivated at 30℃ and 200 rpm. Then precultures were inoculated in 5 ml TY medium supplemented with chloramphenicol with a starting OD_{600} of 0.005 and grown at 30℃ with 200 rpm shaking for 48 h. After that, cultures were allowed to stand at room temperature without shaking. After 42 h pictures were taken.

7. Lipolytic activity assay

7.1. Tributyrin plate assay

Strains of *B. glumae* were inoculated on LB agar plates containing 1% Tributyrin (TBT) as an indicator substrate to measure lipolytic activity, according to the method described by Beisson (Beisson *et al.*, 2000). LB agar was prepared and melted using a microwave. After the addition of TBT, an ULTRA TURRAX® T18 basic homogenizer (IKA WORKS Inc., Wilmington, NC, USA) was employed to homogenize the medium for 3-4 min. Then agar was autoclaved at 121℃ for 20 min and poured into petri dishes immediately to avoid TBT drops. 15 µl of an overnight culture with 1.0 value of OD_{600} was spotted on the agar plate and incubated at 30℃

for 3 days until visible growth. The radius of halo zone around colonies was measured.

7.2. 4-nitrophenol ester assay

4-nitrophenol ester assay was performed to monitor lipolytic activity according to the method of Nawani *et. al*. with some modifications (Nawani *et al.*, 2006). 435 µl of 0.1 M phosphate buffer (pH 8.0), 25 µl of 1 mM octanoate (Sigma Aldrich, Munich, Germany) and 40 µl of culture supernatants were combined and reacted at 37℃ for up to 20 min. 1 ml ethanol was added to stop the reaction, followed by a centrifugation step at 2℃, 13,000 rpm for 5 min. The extinction of supernatant was measured at 405 nm with a SmartSpec™ Plus Spectrometer (Bio-Rad Laboratories, Munich, Germany). The relative lipase activity was calculated as a ratio of OD_{405}/OD_{600}.

8. Virulence assay

8.1. Colony variation assay

Colony variation assay was performed in Casamino acid-Peptone-Glucose (CPG) agar plates containing tetrazolium chloride (TZC) to evaluate the virulence of *B. glumae* strains (Kelman 1954; Kato et al. 2013). Preparation of CPG-agar was given below.

CPG-TZC-agar (500ml)

Casamino acid	0.5g
Peptone	5g
Glucose	2.5g
Agar	8.5g
H_2O_{bidest}	ad 500ml
	Autoclaved (121℃, 20 min)
	Cool the medium to 55℃
TZC (1%)	2.5ml

The overnight culture of *B. glumae* strains was diluted to an OD_{600} of 0.001. Then, 50 µl of diluted bacteria culture was plated on TZC agar plate. After overnight incubation at 30℃, pictures of single colonies were taken and analyzed.

8.2. Onion maceration assay

The onion maceration assay was performed to identify the plant maceration ability of *B. glumae* strains as described previously (Chen *et al.*, 2012; Jacobs *et al.*, 2008; Karki *et al.*, 2012). Spanish golden onions used in this study were purchased from a supermarket REWE in Hamburg. The bulb scales were separated with a sterile razor blade and diced into pieces with an approximate size of 2.5×2.5 cm. A 2 mm of fissure in the middle of each piece of onion bulb scale was made by a 1 ml sterile tip. Each scale was transferred into a 15 mm × 100 mm petri dish, containing 3 layers of pre-moistened filter papers (90 mm in diameter, Whatman No.1). *B. glumae* strains grown on LB agar plate were picked up and suspended in 10 mM $MgCl_2$, 2.5 µl of suspension (1×10^7 cells/ml) was inoculated into the fissure of each onion scale and incubated at 30℃. Pictures were taken after 72 h incubation.

8.2. Virulence assays on rice

Rice seed germination assay was performed to test virulence of *B. glumae* strains (Devescovi et al.). Briefly, seeds of rice (Oryza sativa cv. Baldo) were sterilized with 70% (v/v) ethanol for 5 min, washed once with sterile H_2O_{bidest}, one time in 3% H_2O_2 for 20 min, and three times with sterile H_2O_{bidest}. Then rice seeds were pre-germinated in the suspension of *B. glumae* stains (1×10^7 cells/ml) at 37℃. After 2 days pre-germination, rice seeds were moved into a 15 mm × 100 mm Petri dish containing 3 layers of pre-moistened filter paper (90 mm in diameter, Whatman No.1) to continue germinate under a growth cycle of 16 h light and 8 h dark at 28℃. After 7 days, the germination rate was computed according to International Seed Testing Association rules (Germination %= (Number of germinated seeds/Number of all seeds)×100) (KHARB *et al.*, 1994). After 2 weeks, seedling length of rice seeds was measured using a ruler and pictures were taken.

Rice plant infection assay was performed to further investigate virulence of *B. glumae* strains on the rice spikes (Karki *et al.*, 2012). Rice was planted in a Green House of Hamburg University at 28℃ at night and 35℃ during day time with a 12/12 hour day/night change. At the 30% flowering stage, the cell suspension of *B. glumae* strains (1×10^8 cells/ml) was sprayed

on rice spikes. Each testing contained 10 biological replicates. After 10 days, the spikes were harvested and pictures were taken by a digital camera.

9. Transcriptome analysis of *B. glumae* PG1 and *bgaI* mutants

9.1. Growth curve

Growth curve was generated to analyze growth difference among the parental strain BGPG1 and three *bgaI* mutants BGPG2-4. Bacteria of different strains were pre-grown in 5 ml LB medium from cryogenic cultures at 30℃ and 200 rpm. 100 ml of bacterial suspensions with a starting OD_{600} of 0.005 were prepared by pre-cultures and incubated at 30℃ in a shaking incubator at 200 rpm. Samples were taken every 2 hours for 32 hours and the bacteria growth was determined using optical density data at 600 nm (OD_{600}) by a 1ml cuvette and a SmartSpec™ Plus Spectrometer (Bio-Rad Laboratories, Munich, Germany). Each time point for each strain was measured in triplicate and sterile LB medium used as the reference.

9.2. Preparation of transcriptome samples

Cultures of BGPG1-4 for the transcriptome analysis are summarized in Table 14. To prepare samples for transcriptome analysis, preculture was first established from cryogenic cultures in 5 ml LB medium and cultivated at 30℃ and 200 rpm. After overnight cultivation, a proper volume of preculture was added into 100 ml LB medium to an OD_{600} of 0.005. After 28 hours of incubation at 30℃ and 200 rpm, culture was transferred into falcon with 45 ml for one fraction and chilled on ice, followed by a centrifugation step at 5,000 rpm, 4℃ for 15 min. Supernatant was discarded and cell pellet was directly placed in liquid nitrogen and stored at -70℃. Each strain with three replicates samples and in total 12 samples were shipped to Göttingen Genomics Laboratory of Göttingen University (Göttingen, Germany) for RNA extraction, cDNA library preparation and RNA-seq.

9.3. RNA sequencing analysis of transcriptome samples

(This part was done by using the RNA-seq service from the Göttingen Genomics Laboratory of Georg-August-University Göttingen)

RNA-seq libraries of wild type strain BGPG1 and mutant strains BGPG2-4 were constructed from independent biological triplicates of RNA samples. Total RNA was extracted using the RNeasy Mini Kit (Qiagen, Hilden, Germany). The residual genomic DNA was removed by DNase I (Fermentas, St. Leon-Rot, Germany) treatment. To reduce the amount of rRNA derived sequences, the samples were subjected to rRNA depletion using the RiboZero Magnetic Kit (Epicentre Biotechnologies, Madison, WI, USA). The strand specific cDNA libraries for sequencing were constructed with the NEBNext Ultra directional RNA Library Kit for Illumina (New England Biolabs, Frankfurt/Main, Germany). The obtained cDNA libraries were sequenced using a GAIIx machine (Illumina Inc., San Diego, USA) in single-read mode and running 75 cycles. 32.5 mio to 45.3 mio. raw reads were retrieved for each analyzed sample(Table 14). To assure a high sequence quality, remaining sequencing adaptors were removed and the reads were trimmed with a cut off phred-33 score of 15 by the program Trimmomatic (Bolger *et al.*, 2014). The remaining sequences were mapped with Bowtie 2 (Langmead & Salzberg, 2012) using the implemented end-to-end mode, which requires the entire read align from one end to the other. Differential expression analyses were performed with baySeq (Hardcastle & Kelly, 2010). Genes with a fold-change of ≥ 2.0, a Likelihood value ≥0.9 and an adjusted p-value (p-value was corrected by FDR (false discovery rate) based on Benjamini-Hochberg procedure) ≤ 0.05 were considered as differentially expressed. The trimmed reads have been deposited in the National Center for Biotechnology Information's (NCBI) Sequence Read Archive (SRA) under the Accession No. SRP047507.

9.4. Quantitative real time PCR (qPCR)

(This part of the work was performed by Dr. Andreas Knapp from the institute of molecular enzymtechnology of Heinrich Heine University Düsseldorf)

The qPCR was conducted to verify gene expression patterns obtained from RNA-seq. Firstly, bacterial RNA was isolated from cell pellet of 2 ml culture using RNeasy Mini Kit (Qiagen, Hilden, Germany) according to the instructions of the protocol. DNase I digestion was performed to remove the residual genomic DNA. After that, Maxima First Strand cDNA Synthesis Kit for RT-qPCR (Thermo Scientific, Vilnius, Lithuania) was used to synthetize cDNA from the isolated RNA samples. Real time qPCR was performed to analyze

transcription of candidate genes (Table 12) using the $2^{-\Delta\Delta CT}$ method (Schmittgen & Livak, 2008), and the *rpoD* was used as the internal reference gene to normalize qPCR data. Target mRNA levels were measured in triplicate using the 7900HT Fast Real-Time PCR System" (Applied Biosystems™, Foster City, USA) with Maxima SYBR Green/ROX qPCR Master Mix (Thermo Scientific, Vilnius, Lithuania) following the manufacturer's instructions. Software Primer 3 was used to design the primers (Untergasser *et al.*, 2012). The amount of PCR products was calculated as CT value by the Sequence Detection System (Version 2.3, Applied Biosystems™). PCR efficiencies were determined with the tool "LinRegPCR" (Ruijter *et al.*, 2009). Calculations of exchanged transcript levels were performed and statistically analyzed with the REST© software (Pfaffl *et al.*, 2002). A transcript exchange was assumed to be significantly different from the control sample if $p<0.05$, which was calculated with REST©.

Table 12. Primers used for qPCR.

gene locus	Gene name	qPCR primers
BGL_2c18660	*lipA*	CTATCCGGTGATCCTCGTC
		GAGAGATTCGCGACGTACAC
BGL_2c18670	*lipB*	GTGGCAGACGCGCTATCAAG
		CGTGAAAGTCTGCTGCCTGAG
BGL_2c21380	*rpoD*	GATGACGACGCAACCCAGAG
		GAACGCTTCCTTCAGCAGCA
BGL_2c07470	*rhlA*	TGAAGCCGGAGGCCTATCTC
		TTGCCGATCGTCTCGAACTC
BGL_2c07480	*rhlB*	TACGTGTCGGTGCAGGTGTC
		GTGATGAGCCCCGTCTTCAG
BGL_1c18830	*csy1*	TCGCCGTGCAGAAACTTGGC
		GCAGATGGTTGAGGCGGCTG
BGL_1c18840	*csy2*	TATCGAGGCGCTGCTGGTCC
		TTGCAGCGCCCACATCAACC
BGL_1c01710	*flhA1*	TCAAGCGGATCAAGAGCATCC
		GAGGTTGTCGCGGATATGGA
BGL_1c35020	*flgB2*	CGTTCGCTCGTACCGGCAG
		CGACGTCGCGGGCCTGGTAG

10. Bioinformatics analysis

The following programs and databases were employed for the transcriptome analysis and the sequence analysis of nucleotides and amino acids.

10.1. Program

BiEoEdit	
(Sequence alignment editor)	http://www.mbio.ncsu.edu/bioedit/bioedit.html
Clone Manager Suite 9	
(Edition of sequence files)	Sci-Ed Software (Licensed)
Quality One	
(Gel picture software)	Bio-Rad Laboratories, Munich, Germany
Trimmomatic	
(Reads trimming tool)	http://www.usadellab.org/cms/index.php?page=trimmomatic
Bayseq	
(DEG analysis)	http://www.bioconductor.org
Trav	
(Transcriptome viewer)	http://appmibio.uni-goettingen.de/
Primer 3	
(qPCR primers disign)	http://www.premierbiosoft.com/primerdesign/index.html
Mega 6	
(Phylogenetic analysis)	http://www.megasoftware.net/

10.2. Database

NCBI	http://www.ncbi.nlm.nih.gov/
IMG	http://img.jgi.doe.gov
KEGG	http://www.genome.jp/kegg/kegg2.html
EMBL-EBI	http://www.ebi.ac.uk/
UniProt	http://www.uniprot.org/

III. Results

1. BGPG1 harbors three AHL synthase genes

The motile, rod shaped Gram-negative soil bacterium *Burkholderia glumae* is considered to be a seed-born pathogen that causes panicle blight of rice (Ham *et al.*, 2011; Kim *et al.*, 2004). Strain BGPG1 is used for the production of two biotechnologically relevant enzymes, a lipase similar to the one produced by *P. aeruginosa* and a butyneol I esterase, used by the company BASF SE for the synthesis of pharmaceuticals (Balkenhohl *et al.*, 1997; Hauer *et al.*, 2002).

Table 13. Homology analysis of AHL synthase in *B. glumae* isolates.

***B. glumae* strains**	***bgaI1***	***bgaI2***	***bgaI3***
BGPG1	+	+	+
336gr-1	+	-	-
BGR1	+	-	-
LMG2196	+	-	-
AU6208	+	-	-
3252-8	+	-	-

"+": found; "-": not found.

The newly established genome of BGPG1 revealed the presence of three distinct *luxI* homologous genes, named as *bgaI1-3* (Knapp *et al.*, 2015; Voget *et al.*, 2015). Among them, amino acids sequence of *bgaI1* is highly homologous to the *tofI* gene of *B. glumae* BGR1 (95%) identity on the amino acid level) (Figure 12-13); *bgaI2* has a 53% identity to AHL synthase of *B. thailandensis* (WP_006029278) (Figure12 and 14); *bgaI3* has a 46% identity to AHL synthase of *B. mallei* (WP_004195479) (Figure 12 and 15). Furthermore, these three *bgaI* genes were blasted against the genome sequences of other five *B. glumae* strains and results indicated that the orthologous genes *bgaI1* (95% identity on amino acids level) is highly conserved in each strain, but *bgaI2* and *bgaI3* are only present in BGPG1 (Table 13). Further comparative genetic analysis showed that no other autoinducer synthase genes are present in the genome of BGPG1. These observations suggested that BGPG1 modulates its lifestyle through three QS regulatory circuits, which could be much more complicated than other *B. glumae* isolates.

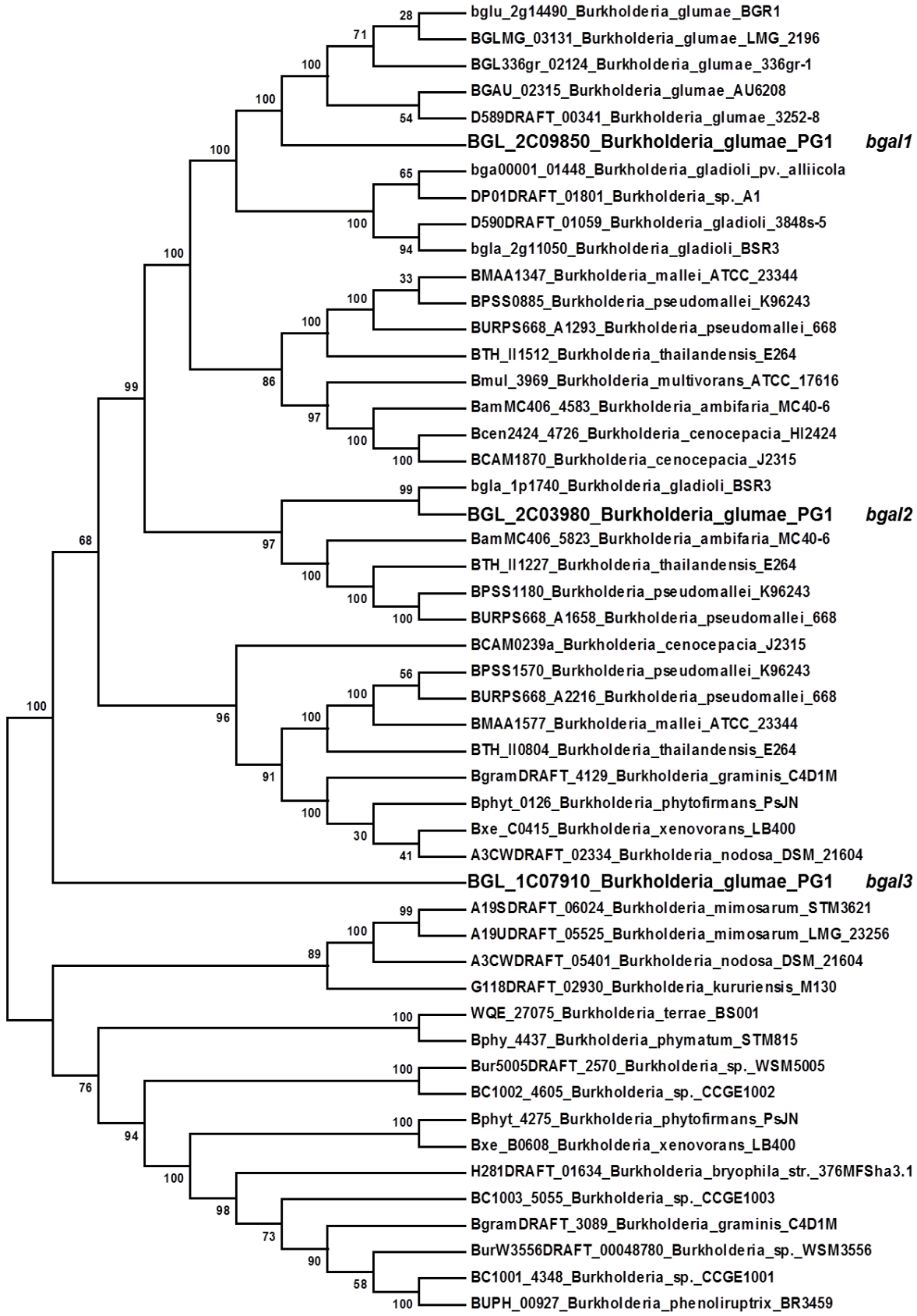


Figure 12. Phylogenetic analyses of AHL synthases in the genus *Burkholderia* based on DNA sequences. The genetic tree was established using Maximum Composite Likelihood method in the MEGA 6 software package. The *bgal1-3* genes of BGPG1 are marked in bold.

```
                    10          20          30          40          50          60
                    ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
BGPG1_BgaI1         MQTSVHEAGR LPSHIAADLG SYRYRVFVEQ LGWKLPSEDE KFERDQYDRD DTVYVLGRDA
BGR1_TofI           ...F...... ..A....E.. .......... ...Q...... .......... ..........
BM_BmaI1            .R.F..GD.. ...DL..... L..HG..... .......AS. G......... .....FA..D
BP_BpsI1            .R.F..GD.. ...DL..... L..H...... .......AS. G......... .....FA..D
BT_BtaI1            .R.F..GD.. ...DL..... L..H.I.... .......AS. G......... .....FA..D

                    70          80          90          100         110         120
                    ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
BGPG1_BgaI1         NGEICGCARL LPTTRPYLLQ EVFPHLLAED NPAPRSTDVW ELSRFAATPE EGAEASSLAW
BGR1_TofI           .......... .......... ........DE H.....AH.. .......... ...D.G....
BM_BmaI1            D......... .........K .L..T.V.Q. M.L.Q.AA.. .......NA. DP.GGGNP..
BP_BpsI1            D......... .........K .L..T.V.Q. M.L.Q.AA.. .......NA. DP.GGGNP..
BT_BtaI1            G......... .........K .L..A.V.H. M.L.Q.AA.. .......NA. DP.GTGNP..

                    130         140         150         160         170         180
                    ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
BGPG1_BgaI1         SVRPMLAAAV ECAARRGARQ LIGVTFCSIE RLFRRIGVHA HRAGAPVSID GRMVVACWID
BGR1_TofI           .......... .......... .......... .......... .......... ..........
BM_BmaI1            A.......V. .....L..K. ......L.M. .......... ....PAQQ.. ..........
BP_BpsI1            A.......V. .....L..K. ......L.M. .......... ....PAQQ.. ..........
BT_BtaI1            A.......V. .....L.... ......L.M. .......... ....PAQQ.. ..........

                    190         200
                    ....|....| ....|....| ...
BGPG1_BgaI1         IDAQTLAALD LDPALCASQP EAA
BGR1_TofI           .......... .......... ...
BM_BmaI1            L......... ...L...PPA ...
BP_BpsI1            L......... ...L...PPA ...
BT_BtaI1            L......... ...L...PPA ...
```

Figure 13. Multiple sequence alignments of BgaI1 with another *Burkholderia* AHL synthases. The amino acid sequences of AHL synthases used here were separately collected from *Burkholderia glumae* PG1 (BGL_2c09850), *Burkholderia glumae* BGR1 (bglu_2g14490), *Burkholderia mallei* ATCC 23344 (BMAA1347), *Burkholderia pseudomallei* 1026b (BP1026B_II0971) and *Burkholderia thailandensis* E264 (BTH_II1512).

```
                    10          20          30          40          50          60
                    ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
BGPG1_BgaI2         MMTTIRRTGD DLAELFR~PL AEYRHAVFVE KLGWPLAVRD GLELDQFDGA GTRYLIGRDG
BT_BtaI2            .ID.TVISAA Q.DSTVKAA. GN..R.I.I. ......PLV. ...I....RP D.I.VV.KTE
BP_BpsI2            .ID.TVISAA Q.DSTVKAA. GN..R.I.I. ......PLV. ...I....RP D.I.VV.KTE

                    70          80          90          100         110         120
                    ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
BGPG1_BgaI2         NGAICGCARL LPTRGAYLLS DVFASLLDGA APPHDDRVWE LSRFAVTHPA DAGRAIIDVD
BT_BtaI2            S.D....... ...TRP...G ...PD.MGD. ...CSAH... I...SSSILS GGPD.LRQAH
BP_BpsI2            S.D....... ...TRP...G E..PD.MGD. ...CSAH... I...SSSILS GGPD.LRQAH

                    130         140         150         160         170         180
                    ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
BGPG1_BgaI2         AATRAMLGAS VRLAAECGAE RLITVSPLGI ERLLLRMGVD AHRAGRPQRI GGKPIFACWI
BT_BtaI2            RN..IL.AKI ..F.QAA.VK ........AV ....N.LK.H I....P.RL. D...V.....
BP_BpsI2            RN..IL.AKI ..F.QAA.VK ........AV ....N.LK.H I....P.RL. D...V.....

                    190         200         210
                    ....|....| ....|....| ....|....| ....|..
BGPG1_BgaI2         ELNRQTAEAL HLAPPPGANR LPPPAERAVR PHLGARA
BT_BtaI2            .VDDI.LQ.. DIE.AADS~~ ~~~~.AG.L. HS
BP_BpsI2            .VDDI.FQ.. DIE.AADS~~ ~~~~.AG.L. HS
```

Figure 14. Multiple sequence alignments of BgaI2 with another *Burkholderia* AHL synthases. The amino acid sequences of AHL synthases used here were separately collected from *Burkholderia glumae* PG1 (BGL_2c03980), *Burkholderia pseudomallei* 1026b (BP1026B_II1251) and *Burkholderia thailandensis* MSMB43 (A33KDRAFT_05783).

```
                    10          20          30          40          50          60
               ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
BGPG1_BgaI3    ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~
BP_ bpsI3      ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~
BT_BtaI3       MRTPPPSSPG CAAPGARAVR CGTAAFSCCR TAFETAHASC QRSPPLRDRT RAAAHANDRS
BM_BmaI3       ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~

                    70          80          90          100         110         120
               ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
BGPG1_BgaI3    ~~~~~~~MKA VSGSRASFEP ALLAQLGRFR HGVFVKHLKW DLPMVSANDE FEWDEFDRAD
BP_ bpsI3      ~~~~~~~MSYI IA.RLNELP. HVQTD..AY. YD...RR.G. TIAGH.LDEH A......GPS
BT_BtaI3       IGEPSAMSYI IA.RLNELP. HVQTD..AY. YD...RR.G. TIAGRAHDGH A......GPS
BM_BmaI3       ~~~~~~~MSYI IA.RLNELP. HVQTD..AY. YD...RR.G. TIAGH.LDEH A......GPS

                    130         140         150         160         170         180
               ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
BGPG1_BgaI3    AVYVVLRSRD GDVRACARLL ATTAPCLMHK LLPDAQPEAP DPR~~~VWEL SRVAVAQRAA
BP_ bpsI3      TIH..ALDDA REICGY.... P..G.Y.LRD VFAHLLGSS. A.QSPA...M ..F.ASR.RR
BT_BtaI3       TIH..ALDDA RE.CGY.... P..G.Y.LRD VF.HLLGSS. A.QSPA...M ..F.ASR.RR
BM_BmaI3       TIH..ALDDA REICGY.... P..G.Y.LRD VFAHLLGSS. A.QSPA...M ..F.ASR.RR

                    190         200         210         220         230         240
               ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
BGPG1_BgaI3    AEAGDGRDAM QTLLAAVIEA ARARGISRLI GVAAPAMMRL YRKKGFRLVP EGKAFVLAGE
BP_ bpsI3      SAT~EREPLG MAFFPS.LTV .ASL.AT.VV ..MT.SIE.. ..RS.IA.HR L.N.MPG..G
BT_BtaI3       RAT~EREPLG MDFFPS.LTV .ASL.AT.VV ..MTLSIE.. ..RS.IA.HR L.S.MPS..G
BM_BmaI3       SAT~EREPLG MAFFPS.LTV .ASL.AT.VV ..MT.SIE.. ..RS.IA.HR L.N.MPG..G

                    250         260
               ....|....| ....|....| ....|....
BGPG1_BgaI3    SLMRFSLYVF DGRAAERARL AG
BP_ bpsI3      ..SAC.IDLP RLAF.PLG.K QCAACLAMH
BT_BtaI3       ..SAC.VELP CLAF.PLG.K QCASCLSMH
BM_BmaI3       ..SAC.IDLP RLAF.PLGLK QCAACLAMH
```

Figure 15. Multiple sequence alignments of BgaI3 with another *Burkholderia* AHL synthases. The amino acid sequences of AHL synthases used here were separately collected from *Burkholderia glumae* PG1 (BGL_1c07910), *Burkholderia mallei* ATCC 23344 (BMAA1577), *Burkholderia pseudomallei* 1026b (BP1026B_II1673) and *Burkholderia thailandensis* E264 (BTH_II0804).

2. Characterization of *bgaI1-3* genes

2.1. Cloning of *bgaI1-3* genes

AHL synthase genes, *bgaI1-3,* were PCR-amplified using genomic DNA of BGPG1 as the template with specific primers listed in Table 5. Agarose gel electrophoresis was used to determine the accuracy and quality of PCR products. The sizes of *bgaI1-3* were 1032 bps, 943 bps and 1117bps, respectively (Figure 16A). After purification with a Gel/PCR DNA Fragments Extraction kit (II 4.6), each PCR product was inserted into pDrive vector separately (II 4.7.2.1) to produce pDrive::*bgaI1*, pDrive::*bgaI2* and pDrive::*bgaI3*. Then they were transformed into *E. coli* DH5α by heat shock and selected on LB agar plates containing ampicillin, IPTG and X-Gal (II 4.9.1.2). White colonies supposed to carry these *bgaI* genes were inoculated into 5 ml LB medium supplemented with ampicillin. Colony PCR (II 4.8.3) and

DNA sequencing (II 4.10) were used to confirm the presence of each AHL synthase gene.

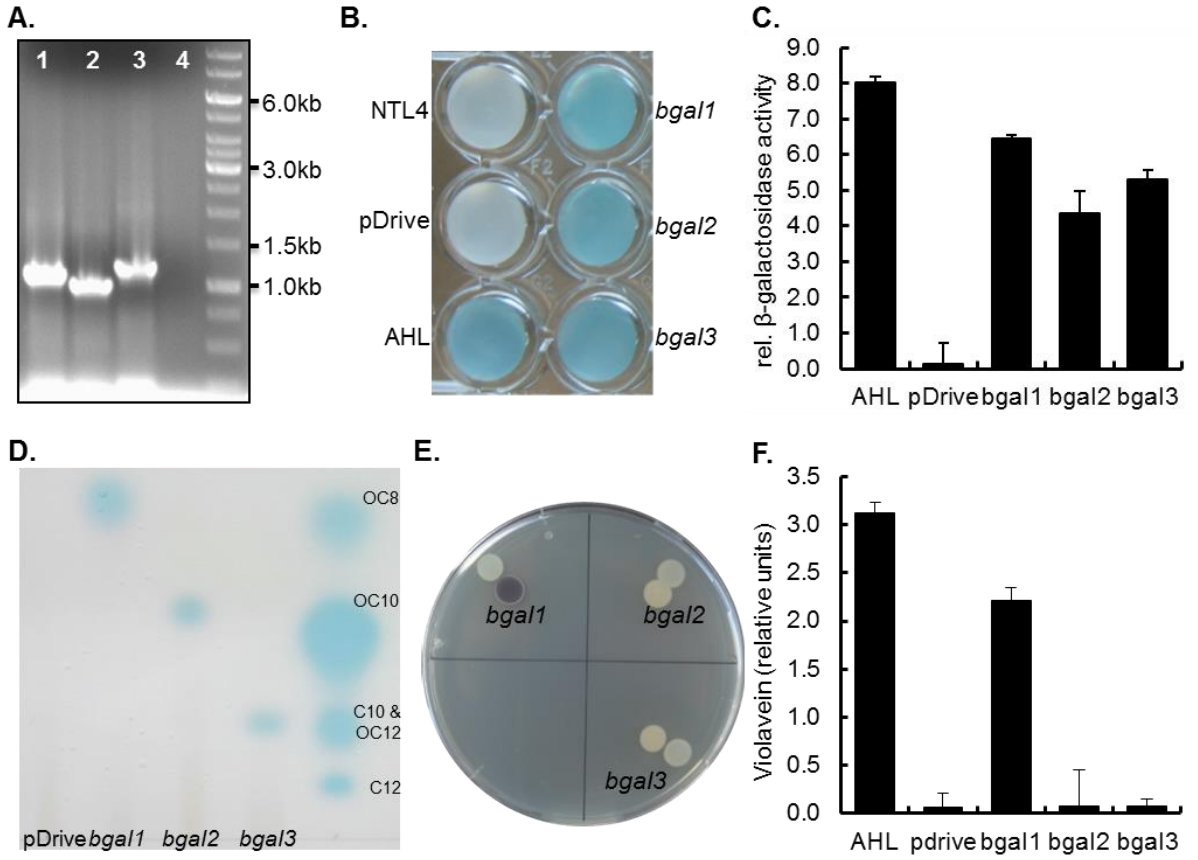


Figure 16. Characterization of three AHL synthase genes *bgal1-3*. (A) PCR products of *bgal1-3* genes were electrophoresed on 1% agarose gel. Lane 1: *bgal1*. Lane 2: *bgal2*. Lane 3: *bgal3*. Lane 4: negative control. **(B)** Verification of AHL production by *bgal1-3* clones using AT soft agar screening together with *A. tumefaciens* NTL4. **(C)** Verification of AHL production by *bgal1-3* clones with ONPG assay using *A.tumefaciens* NTL4. **(D)** AHL profiles of *bgal1-3 E. coli* clones in TLC plate and AHL detection by overlay assay using *A. tumefaciens* NTL4. Lane 1: pDrive. Lane 2: *bgal1*. Lane 3: *bgal2*. Lane 4: *bgal3*. Lane 5: OC8 (3-oxo-C8-AHL), C10 (C10-AHL) and OC10 (3-oxo-C10-AHL), C12 (C12-AHL) and OC12 (3-oxo-C12-AHL). **(E)** Verification of AHL production by *bgal1-3* clones using *C. violaceum* CV026 on LB agar plate. **(F)** Verification of AHL production by *bgal1-3* clones through measuring violacein production in liquid LB medium using *C. violaceum* CV026.

2.2. AHL production of *bgal1-3* clones verified by AHL reporters

2.2.1. AHL production of *bgal1-3* clones verified by *A. tumefaciens* NTL4

AT soft agar screening (II 5.2) and ONPG assay (II 5.3), both based on *A. tumefaciens* NTL4 reporter system, were employed in this study to detect AHL production. The principle of AT soft agar screening assay is based on the degradation of X-Gal in the agar by β-galactosidase enzyme. Simply, exogenous AHL molecules permeate through AT soft agar and promote

transcription of the *traG*::*lacZ* fusion in *A. tumefaciens* NTL4 (Shaw *et al.*, 1997). The product of *lacZ* gene promotes the release of β-galactosidase enzyme which then cleave the colorless X-Gal in AT soft agar to produce a characteristic blue dye, 4-chloro-3-brom-indigo (Shaw *et al.*, 1997). In this experiment, cultures of *bgaI1-3* clones were loaded onto soft agar plates respectively as exogenous AHL resource. 10^{-6} M C8-AHL solution was used as the positive control. Culture of *E. coli* harboring an empty pDrive vector was used as the negative control. Results were shown in Figure 16B. Cultures from *bgaI1-3* clones were all able to change the AT soft agar to blue with a density comparable with positive control, suggesting all *bgaI1-3* have the ability to produce AHL molecules. The detailed profiles of AHL produced by each gene were further characterized by TLC assay. As shown in Figure 16D, AHL synthesized by *bgaI1-3* corresponds to standard of 3-oxo-C8-AHL, 3-oxo-C10-AHL and C10-AHL or 3-oxo-C12-AHL, respectively.

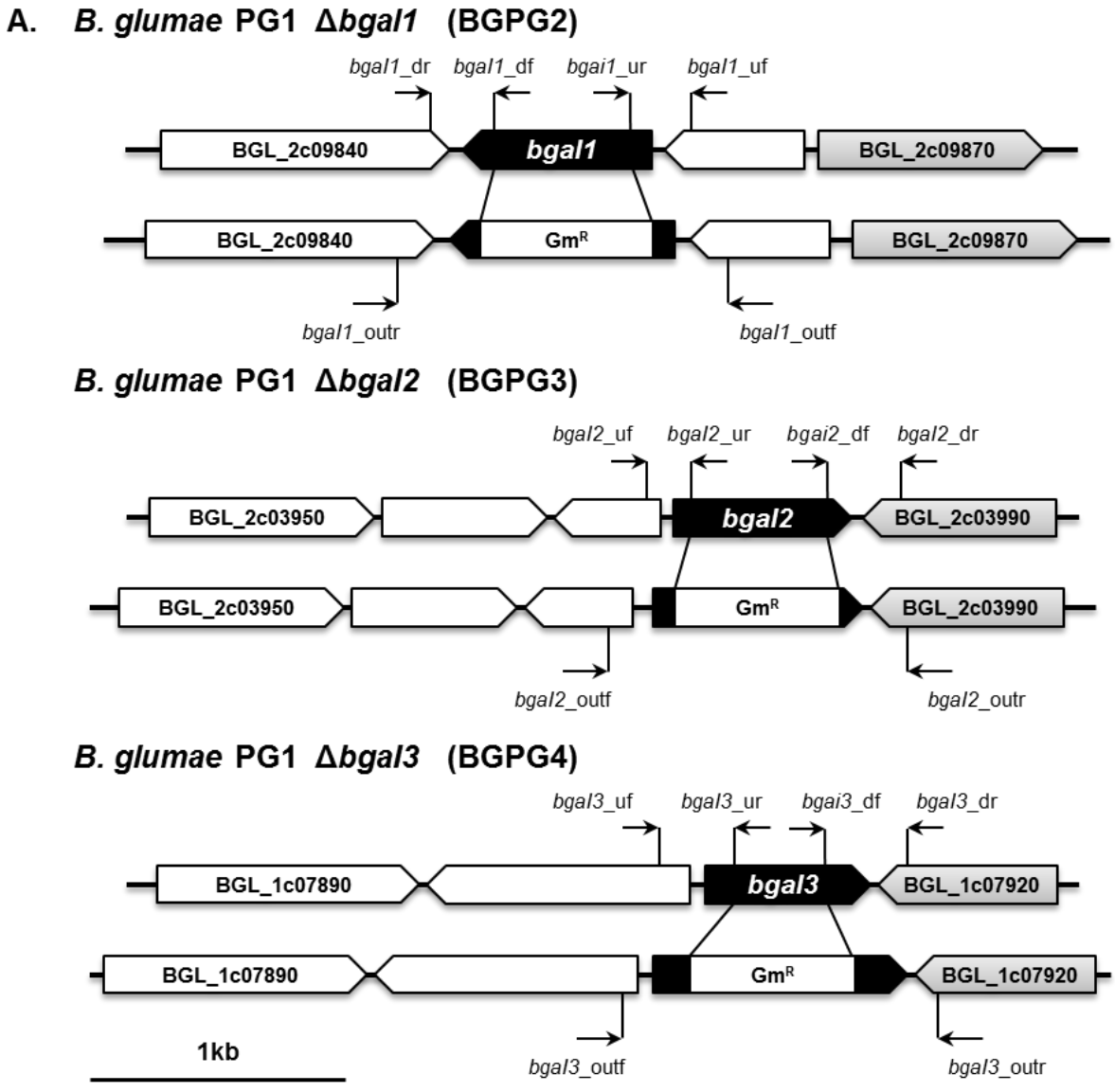
ONPG assay was performed to detect AHL molecules in liquid solution. The principle is the same as the AT soft agar screening assay. In the presence of exogenous AHLs, the colorless indicator ONPG can be degraded by β-galactosidase enzyme to produce a yellow end-product, *O*-nitropheno (ONP) (Lederberg, 1950; Shaw *et al.*, 1997). Therefore, the concentration of exogenous AHLs can be detected by measuring the yellow density at OD_{420} and calculating the OD_{420}/OD_{600} ratios (Figure 16C). In this experiment, cell-free extracts of *bgaI1-3* clones were prepared as described in II 5.1 and used as exogenous AHLs. 10^{-6} M C8-AHL solution was used as positive control and the empty pDrive vector used as negative control. Results were shown in Figure 16C. It turned out that all *bgaI1-3* clones were able to produce a high level of ONP which was comparable with the positive control. The amount of ONP yielded by each culture was about 30-fold, 28-fold and 51-fold higher than negative control, respectively. These results are consistent with the AT soft agar screening assay and confirmed that all *bgaI1-3* clones have a strong capacity of AHLs production.

2.2.2. AHL production of *bgaI1-3* clones verified by *C. violaceum* CV026

C. violaceum CV026 was used to investigate the acyl chain length of AHLs synthesized by each gene. Strain CV026 is a violacein negative mutant strain derived from the wild strain *C.*

violaceum (Blosser & Gray, 2000; Ravn *et al.*, 2001), which can not produce any detectable violacein due to the mutation of AHL synthase gene. However, the production of violacein can be restored by exogenous AHLs with an acyl chain containing 6 or 8 carbons. Therefore, it is a useful indicator to show the acyl chain length of AHLs. Normally, violacein can be qualitatively detected on agar plates or quantitatively detected in liquid by a spectrophotometry (II 5.4).

To perform this assay on agar plates, 5 µl cultures of *bgaI1-3* clones were dropped onto LB agar plates separately, then CV026 strain was dropped parallelly with each of them (Ravn *et al.*, 2001). After overnight incubation at 30℃, purple pigment was produced by CV026 strain in the presence of *bgaI1* clone but not other two clones (Figure 16E).



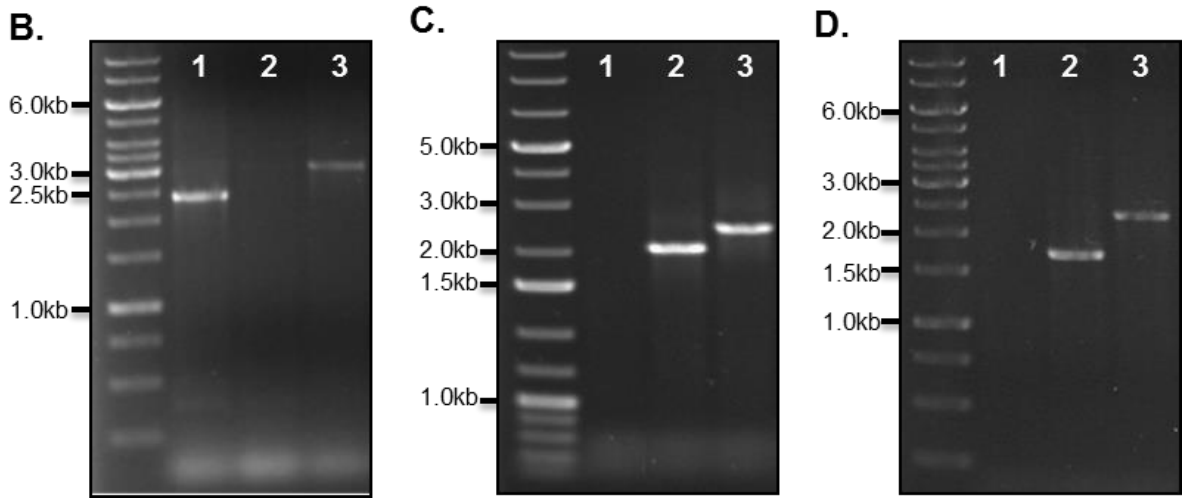


Figure 17. Construction of three AHL synthase deletion mutants of BGPG1. (A) Physical maps of *bgaI1-3* and their deletions and flanking regions. Black colored arrows show the *bgaI1-3* genes; grey colored arrows indicate their putative regulator genes; white arrows are flanking ORFs. The deletion region was located from 1,237,902 bp to 1,238,222 bp (chromosome 2) for *bgaI1*, 486,784 bp to 487,479 bp (chromosome 2) for *bgaI2* and 899,154 bp to 899,509 bp (chromosome 1) for *bgaI3*. Gm^R gene was employed as antibiotic resistance selection marker. (B-D) Confirmation of *bgaI1-3* gene deletion in BGPG2-4 by PCR. **(B)** Lane1: BGPG1. Lane 2: negative control. Lane 3: BGPG2. **(C)** Lane 1: negative control. Lane 2: BGPG1. Lane 3: BGPG3. **(D)** Lane 1: negative control. Lane 2: BGPG1. Lane 3: BGPG4.

To quantitatively detect violacein production, CV026 was inoculated in liquid LB medium, cell-free extracts from *bgaI1-3* clones were added separately into the medium. After 16 hours incubation at 30℃ with shaking, 10% sodium dodecyl sulfate (SDS) was added to lyse the cells, 900 µl of water-saturated butanol was added to extract the pigment violacein. The harvested violacein was measured at OD_{585} by a SmartSpec™ Plus Spectrometer (Bio-Rad Laboratories, Munich, Germany). The relative violacein amount was calculated as the ratio of OD_{585}/OD_{600}. 5 µl of 10^{-6} M C8-AHL in ethyl acetate was used as the positive control. The violacein production by *bgaI1* clone was almost 30-fold higher than by negative control (Figure 16F). However, violacein production by *bgaI2-3* clones was undetectable (Figure 16F), which was consistent with the results obtained from agar plate assay. Taken together with these data, it can be concluded that all *bgaI1-3* clones are able to produce AHL molecules, only the AHL produced by *bgaI1* clone is able to restore production of violacein. This suggested AHL produced by *bgaI1* clone has an acyl chain with 6 or 8 carbons, and AHLs produced by *bgaI2-3* clones have acyl chains longer than 8 carbons.

3. Construction of *bgaI1-3* deletion mutants

To identify functions of these three AHL synthase genes in the genome context, single mutant derivatives from BGPG1 with deletion of *bgaI1*, *bgaI2* or *bgaI3* were generated with pNPTS138-R6KT system by double-crossover homologous recombination in the flanking regions of targeted genes as show in Figure 17A. Gm^R gene was employed as antibiotic resistance selection marker. Putative strains of BGPG2 (Δ*bgaI1*), BGPG3 (Δ*bgaI2*) and BGPG4 (Δ*bgaI3*) were obtained. Each deletion mutant was verified by sequencing and PCR with specific primers corresponded to the flanking sequences of deletion region. According to their DNA constructs, the sizes of PCR-amplified fragments should be 3kb for BGPG2 (Figure 17B), 2.5kb for BGPG3 (Figure 17C) and 2.1kb for BGPG4 (Figure 17D), and the size differences between parent strain and each mutant strain were accorded with expectations.

4. AHL production of BGPG1-4 verified by AHL reporters

4.1. ONPG assay and violacein production assay

It was shown above that all *bgaI1*, *bgaI2* and *bgaI3* genes had a strong capacity to produce AHL molecules. Therefore, it was predicted that AHL production should be decreased after deleting these AHL synthase genes. To test this prediction, ONPG and violacein production assays were carried out again. Results showed there was a 20%, 38% and 48% reduction of ONP production by BGPG2, BGPG3 and BGPG4 respectively compare to wide type BGPG1 (Figure 18C). Above experiments showed that only *bgaI1* can restore the violacein production of CV026 strain. It was confirmed again here that only BGPG2 without *bgaI1* gene lost the ability to restore violacein (Figure 18A-B).

4.2. TLC overlay assay

To obtain a detailed AHL profile of the Δ*bgaI* mutants, TLC overlay assay was performed using *A. tumefaciens* NTL4 (Shaw *et al.*, 1997). As shown in Figure 18D, the parental strain BGPG1 had three AHL signal spots. The highest one has the same position as the standard of 3-oxo-C8-AHL and is absent from the mutant BGPG2, suggesting that *bgaI1* synthesize 3-oxo-C8-AHL. This data is consistent with the results of violacein production assay. The

middle one is corresponding with 3-oxo-C10-AHL and is absent from the mutant BGPG3, suggesting that AHL synthesized by *bgaI2* gene could be 3-oxo-C10-AHL. The lowest one has the same position as C10-AHL or 3-oxo-C12-AHL and is absent from the mutant BGPG4, suggesting that AHL synthesized by *bgaI3* gene could be C10-AHL or 3-oxo-C12-AHL. Through comparing the positions of signal spots of parental strain and mutants, it can be concluded that AHLs synthesized by *bgaI1*, *bgaI2*, *bgaI3* are different in profiles.

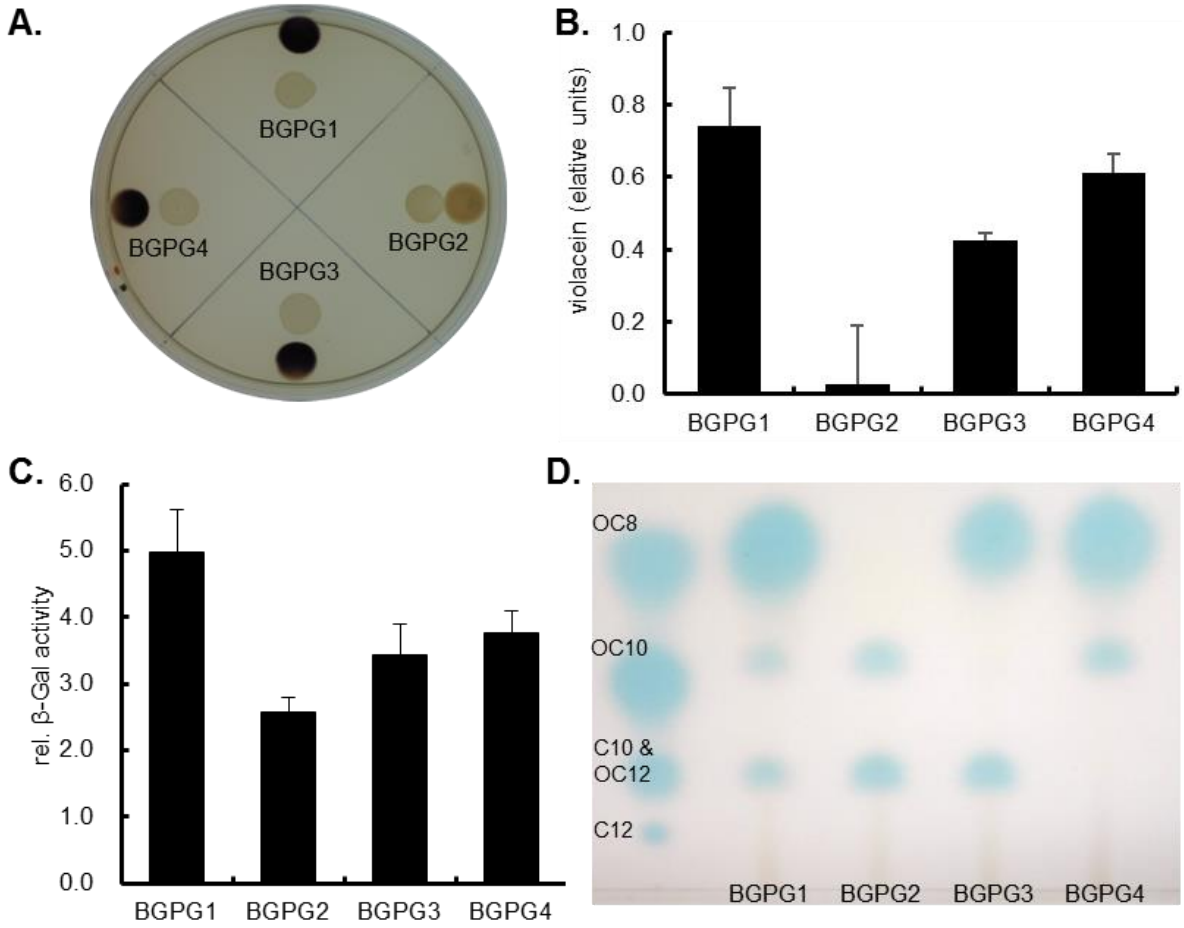


Figure 18. Verification of AHL production by BGPG1-4 using AHL reporters. **(A)** Verification of AHL production by BGPG1-4 using *C. violaceum* CV026 on LB agar plate. **(B)** Verification of AHL production by BGPG1-4 through measuring violacein production using *C. violaceum* CV026. **(C)** Verification of AHL production by BGPG2-4 with ONPG assays using *A. tumefaciens* NTL4. **(D)** Differentiation of AHL profiles of BGPG1-4 in TLC plate by overlay assay using *A. tumefaciens* NTL4. Lane 1: OC8 (3-oxo-C8-AHL), C10 (C10-AHL) and OC10 (3-oxo-C10-AHL), C12 (C12-AHL) and OC12 (3-oxo-C12-AHL). Lane 2: BGPG1. Lane 3: BGPG2. Lane 4: BGPG3. Lane 5: BGPG4.

4.3. Complementation analysis of BGPG2 using violaecin production assay

Complementation assay with *bgaI1* was performed using plasmid pBBR1MSC-2::*bgaI1*. It is

shown in Figure 19, the violacein production in AHL reporter CV026 cannot be rescued by the cell-free extract of BGPG2. After complementation by conjugating the plasmid pBBRMCS-2::*bgaI1* in *trans*, the production of violacein by strain CV026 was rescued by cell-free extract of *B. glumae* PG2c (Figure 19). These results demonstrated that the *bgaI1* deletion mutant was constructed successfully and further confirmed that the *bgaI1* gene is responsible for the synthase of AHL with the 8-carbon acyl chain.

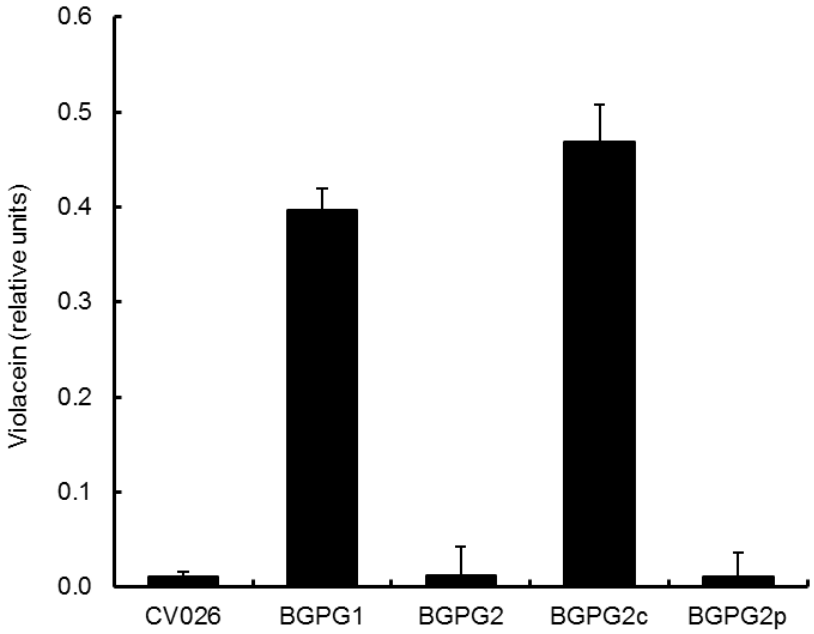


Figure 19. Verification of AHL production by the complemented strain BGPG2c in violacein production assay using reporter strain *C. violaceum* CV026.

5. Roles of BGPG1 QS systems on regulating lipolytic activity

Tributyrin (TBT) was used as an indicator substrate to measure the lipolytic activity of the parental strain BGPG1 and the *bgaI* mutant strains BGPG2-4 on LB agar plates. Lipolytic activity was determined by measuring the size of clearing zones around colonies. Both parental strain BGPG1 and AHL synthase mutants showed obvious clearing zones (Figure 20A). The size of clearing zones by the parental strain was larger than by each mutant, suggesting each deletion mutant has less lipolytic activity compare to wild type BGPG1 (Figure 20B). Lipolytic activity was also quantified by photometer at 405 mm using 4-nitrophenol ester (*p*NP-octanoate) as a substrate. As shown in Figure 20C, BGPG2, BGPG3 and BGPG4 showed a 55%, 70% and 38% reduction of lipolytic activity compared to the parental strain BGPG1. It indicated that each *bgaI* gene plays a role in the lipoliytic activity

in BGPG1.

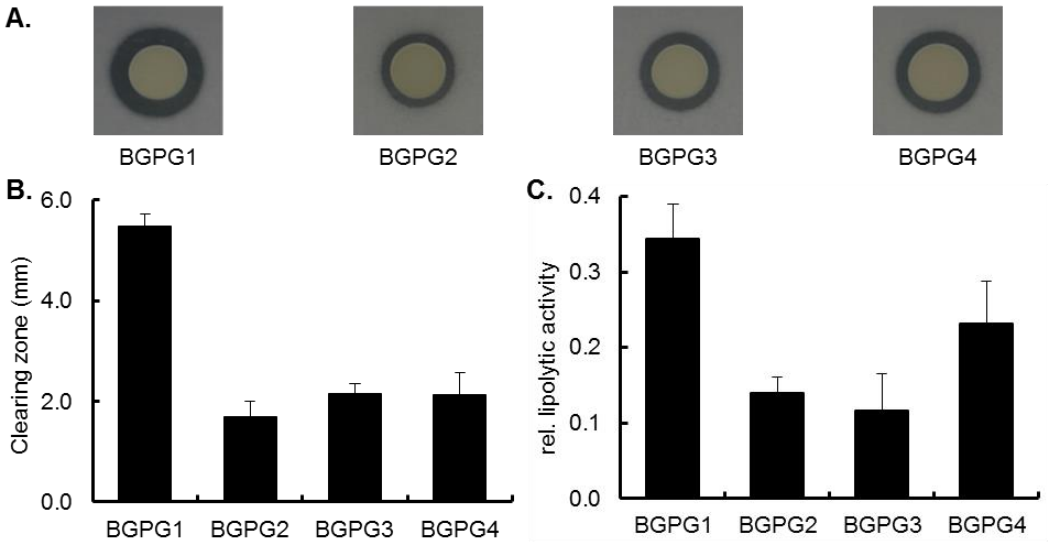


Figure 20. Lipolytic activity assays of BGPG1-4. (A-B) Lipolytic activity assays of BGPG1-4 in TBT agar plate. 15 µl of an overnight culture with 1.0 value of OD_{600} was spotted on a TBT-containing agar plate and incubated at 30℃ for 3 days. The clearing zones around colonies were observed **(A)**, and the radius of halo zone around colonies was measured, data are mean values of five analyzed colonies **(B)**. **(C)** Lipolytic activity assays of BGPG1-4 in liquid medium. Cell supernatant of each strain was reacted with 1 mM octanoate at 37℃ for up to 20 min. After centrifugation, the extinction of supernatant was measured at 405 nm from. The relative lipase activity was calculated as a ratio of OD_{405}/OD_{600}.

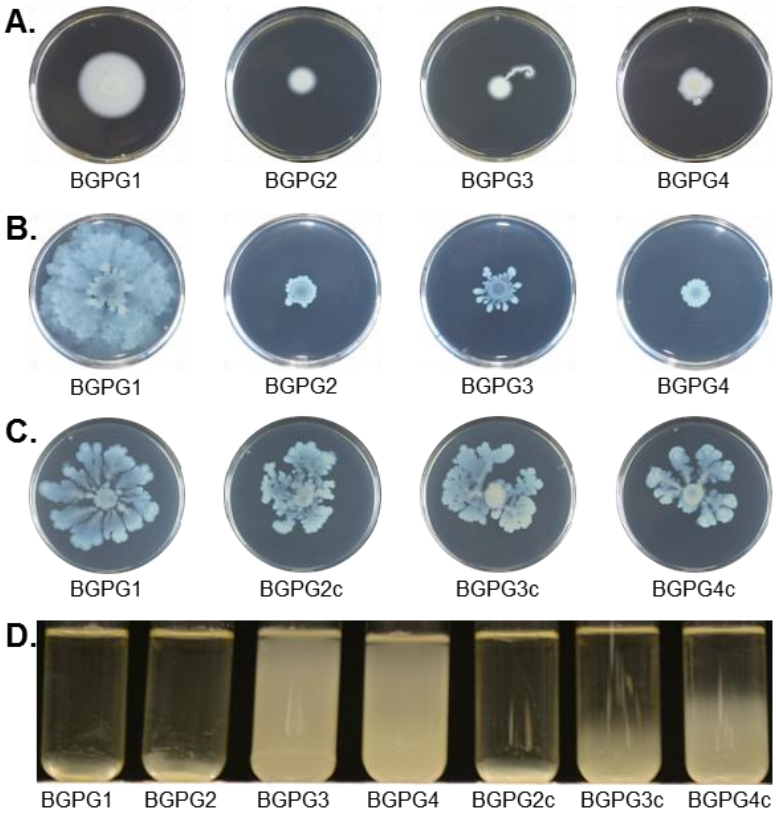


Figure 21. Motility assays of BGPG1-4. (A) Swarming motility assays of BGPG1-4. $1x10^7$ cells of

each strain were inoculated on agar plates containing 0.45% Eiken agar (Eiken, Tokyo, Japan) and incubated for 3 days at 30℃. **(B)** Complementation of swarming motility of BGPG2-4. Each complementation construct based on plasmid pBBR1MCS-2 for *bgaI1-3* was introduced into each mutant strain by conjugation with the donor strain of *E. coli* WM 3064, and then swarming motility was detected. **(C)** Swimming motility assays of BGPG1-4. $1x10^7$ cells of each strain were inoculated on agar plates containing 0.25% Eiken agar (Eiken, Tokyo, Japan) and incubated for 3 days at 28℃. **(D)** Sedimentation assays of BGPG1-4 and three complemented strains, BGPG2c, BGPG3c and BGPG4c, in liquid TY media. Culture of each strain in liquid TY media was allowed to stand at room temperature for 42 h.

6. Roles of BGPG1 QS systems on regulating motility

6.1. Surface motility

Surface motility of *B. glumae* strains was detected by swarming and swimming assays on 0.45% and 0.25% Eiken agar plates with 3 days of incubation at 30℃. It was known AHL-mediated QS positively regulate motility in many bacteria strains. It was expected that deletion of each AHL synthase gene would impair motility of BGPG1. In the swimming test, colonies of parental strain BGPG1 formed a large regular flat circle, whereas each deletion mutant formed a much smaller circle (Figure 21A). In the swarming assay, parental strain BGPG1 occupied the whole plate while each mutant only occupied a much small place (Figure 21B). Furthermore, swarming motility was restored by using the relevant complemented strains (Figure 21C). Both results were consistent with expectations and suggested that each AHL synthase gene positively regulates bacteria motility.

6.2. Sedimentation assay

The sedimentation assay was used to evaluate the difference of motility between the parent strain BGPG1 and three mutants BGPG2-4 in liquid according to the previously described method (Krysciak *et al.*, 2014). The tubes were allowed to stand at room temperature for 42 hours without shaking after cultures of *B. glumae* strains were ready. Within 12 hours, cultures of the parent strain BGPG1 and the mutant BGPG2 settled on the bottom of the glass tube completely, while the mutant strains BGPG3 and BGPG4 did not settle down within 42 hours (Figure 21D). The observed sedimentation phenotype for BGPG3 and BGPG4 was partially restored using the complement strains BGPG3c and BGPG4c (Figure 21D).

7. Roles of BGPG1 QS systems on regulating colony morphology

Colony morphology analysis of the parent strain BGPG1 and mutant strains BGPG2-4 was performed in Casamino acid-Peptone-Glucose (CPG) agar plates containing tetrazolium chloride (TZC) (Kelman 1954; Kato et al. 2013) (Figure 22A). Colonies of the parent strain BGPG1 were light red and oblate, irregular in shape. In contrast to parent strain, colonies of *bgaI* gene deletion mutants BGPG2-4 were dark red and much plumper and regular in shape. Additionally, the size of the colonies of BGPG2 and BGPG3 is slightly smaller than those of BGPG4. Hence, it is easy to distinguish the parental strain and deletion mutants by colony variation on TZC medium.

8. Roles of BGPG1 QS systems on regulating virulence

8.1. Roles of BGPG1 QS systems on regulating plant-maceration

Plant-maceration is one of indicators of bacteria virulence which is regulated by QS system in some other *B. glumae* strains (Jacobs *et al.*, 2008). As shown in Figure 22F, the parental strain BGPG1 produced a clear macerated tissue which is strongly reduced by BGPG3 and nearly disappeared in BGPG2 and BGPG4. It implied that each AHL synthase gene in BGPG1 played a role in plant-maceration (Chen *et al.*, 2012; Jacobs *et al.*, 2008; Karki *et al.*, 2012).

8.2. Roles of BGPG1 QS systems on regulating pathogenicity

To investigate roles of AHL synthase genes on regulating virulence in rice, rice germination and seedling length assays were performed in the presence of parent strain BGPG1 or mutants BGPG2-4. The rice seed germination rate strongly increased after deletion of each AHL synthase gene compared to the parental strain (Figure 22B). And the seedling length of rice seeds was longer in the presence of each mutant than in the presence of parental strain (Figure 22C-D). To further investigate the roles of *bgaI1-3* genes on panicle blight, rice were planted in a Green House and inoculated with suspension of BGPG1 or mutants during 30% flowering period. After 10 days inoculation, no obvious panicle blight in rice spikelets can be observed and the difference of rice spikelets between wide type BGPG1 and each mutant

sprayed rice plants was not significant (Figure 22E). These data collectively indicated that AHL synthase genes positively regulate virulence during rice germination and seedling period but not during rice flowering phase and have no effects on rice panicle blight.

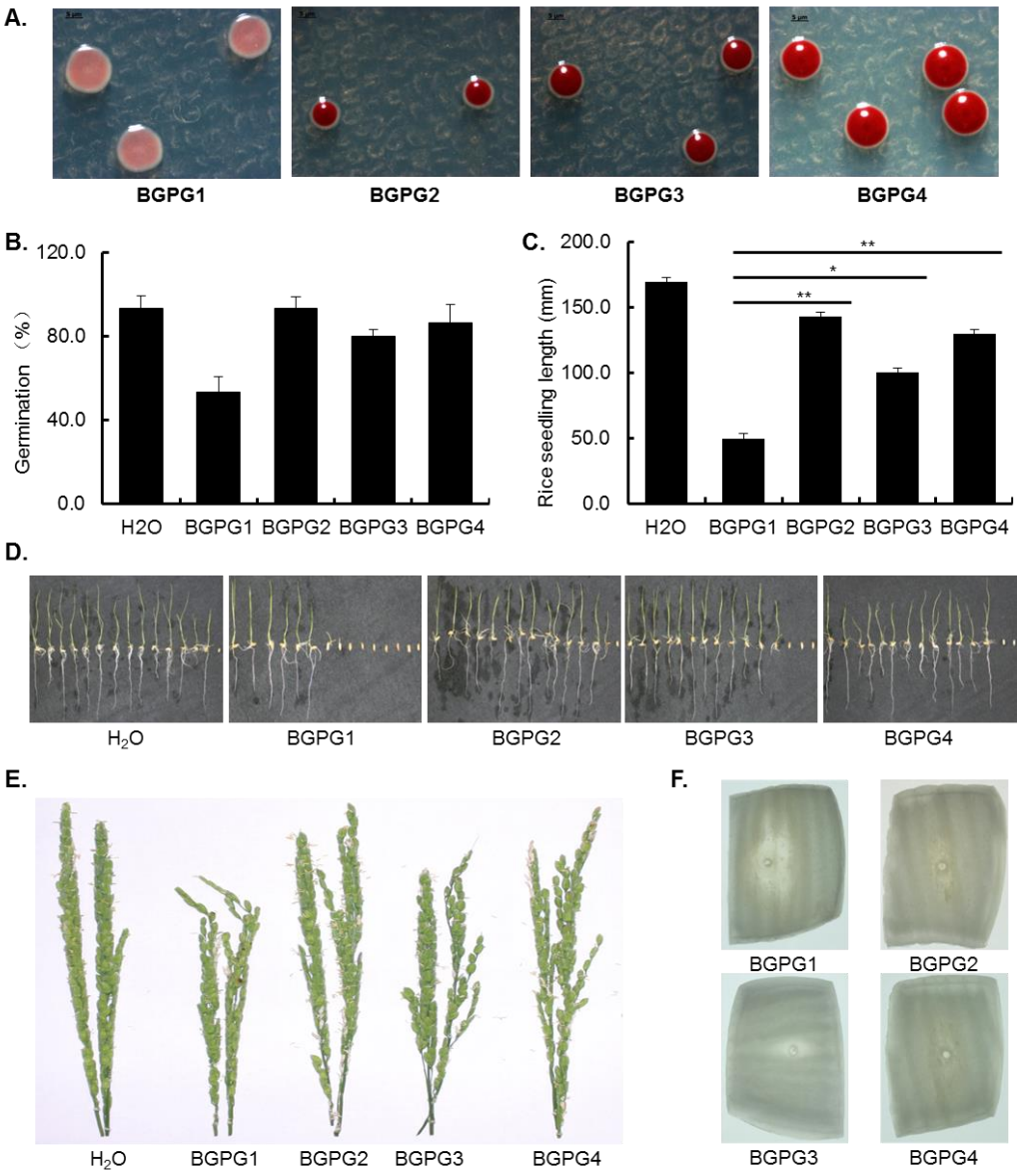


Figure 22. Colony variation on TZC agar plate and plant phenotypes of BGPG1-4. (A) Colony color and morphology analyzed after growing BGPG1-4 on TZC-containing medium for 3 days at 30℃. **(B)** Rice germination assays of BGPG1-4. Rice seeds were germinated in the presence of each strain of BGPG1-4 under a growth cycle of 16 h light and 8 h dark at 28℃. After 7 days, the germination rate was calculated. **(C-D)** Rice seedling assays of BGPG1-4. After 14 days germination, the length of each seedling was measured and recorded **(C)**, pictures were taken for seedlings **(D)**. Data in B and C are mean values of 15 individual seedlings analyzed per treatment. A single asterisk (*) indicates $p < 0.05$; double asterisks (**) indicate $p < 0.01$. **(E)** Pathogenicity assays of BGPG1-4 on rice spikelet. Rice was planted in a Green House at 28℃ at night and 35℃ during day time with a 12/12 hour day/night change. At the 30% flowering stage, the cell suspension of each strain (10^8 cells/ml) was sprayed on spikelets.

After 12 days, the spikes were harvested and pictures were taken by a digital camera. Each testing contained 10 biological replicates. **(F)** Onion maceration assay of BGPG1-4. Slices of onion bulbs were inoculated with BGPG1-4 and incubated for 72 h at 30℃.

9. Transcriptome analysis for investigating QS-regulated genes in BGPG1

9.1. Growth analysis of BGPG1-4

Growth curves of parental strain BGPG1 and AHL synthase deletion mutants BGPG2-4 were determined in 100 ml LB medium. The differences between BGPG1 and mutants BGPG2-4 were not significant (Figure 23). 28 h was chosen as the time point for transcripotome analysis since it is the transition time of all strains from exponential to stationary growth phase. At this time point, all QS regulated processes are speculated to be turned on.

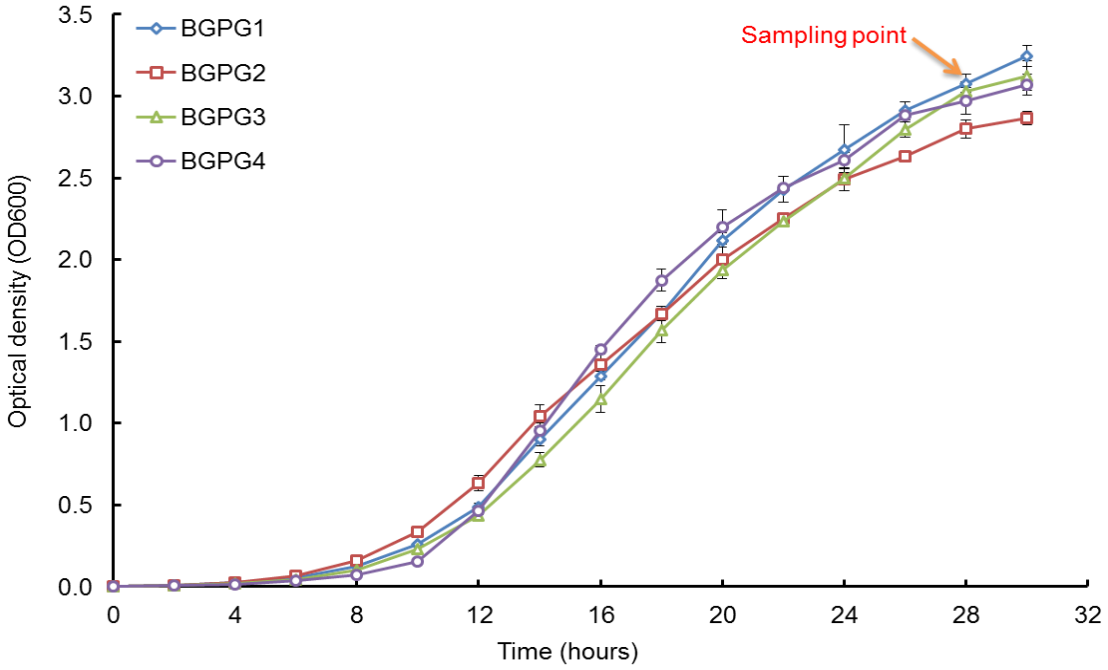


Figure 23. Growth curves of BGPG1-4. The growth of each strain was determined using optical density data (OD) at 600 nm. Bacteria were grown at 30℃ and 200 rpm and samples were taken every 2 hours over a period of 32 hours. Each time point for each strain was measured in triplicate and sterile LB medium used as the reference. Sampling points of transcriptome samples are indicated with orange arrows.

9.2. Quality of RNA-seq data

To further understand the roles of the three AHL synthase genes in BGPG1, genome-wide RNA-seq was performed. The global gene expression pattern of the wild-type BGPG1 and the three AHL synthase mutants (BGPG2-4) was investigated after 28 h incubation at 30℃ based

on the results of growth analysis. The OD_{600} of each sample ranged from 2.8 to 3.07 (Table 14). For each of the four strains three independent biological sample experiments were performed and examined by RNA-seq. Thereby, a total of twelve individual samples were analyzed (Table 14). Alignments were established and for each sample a minimum of 24.6 mio. cDNA reads could be uniquely mapped to the *B. glumae* genome, resulting in 24.6-37.9 million uniquely mapped reads per treatment (Table 14).

Table 14. Overall transcriptome statistics for BGPG1-4.

Sample No.	BGPG1 genotype	OD_{600}	No. of generated reads $X10^6$	No. of uniquely mapped reads $X10^6$
1	wt/BGPG1	3.13	39.3	32.7
2	wt/BGPG1	3.15	45.3	37.9
3	wt/BGPG1	3.09	39.8	34.4
4	Δ*bgaI1*/BGPG2	2.84	43.4	33.0
5	Δ*bgaI1*/BGPG2	2.84	32.5	25.0
6	Δ*bgaI1*/BGPG2	2.81	32.8	28.6
7	Δ*bgaI2*/BGPG3	3.08	44.8	32.1
8	Δ*bgaI2*/BGPG3	3.05	33.5	26.1
9	Δ*bgaI2*/BGPG3	3.07	34.7	26.4
10	Δ*bgaI3*/BGPG4	2.98	42.1	33.4
11	Δ*bgaI3*/BGPG4	2.92	34.6	26.8
12	Δ*bgaI3*/BGPG4	2.91	33.5	24.6

9.3. Identification of QS-regulated genes in BGPG1

Transcriptional profiles of the parent strain BGPG1 and AHL synthase deletion mutants BGPG2-4 from RNA-seq data were analyzed using a method described previously by Nookaew et al (Hardcastle & Kelly, 2010; Nookaew *et al.*, 2012). Differentially expressed genes (DEGs) were selected according to following criterions: fold-change of ≥ 2.0, likelihood

value of ≥ 0.9, and FDR value of ≤ 0.05 (Table 18). Only values that complied with these three requirements were used for subsequent analyses. In comparison with wild-type BGPG1, 481 genes were differentially expressed in BGPG2, 213 in BGPG3, and 367 in BGPG4. Interestingly, only a minor set of 78 genes was co-regulated in all three mutants (Figure 24A).

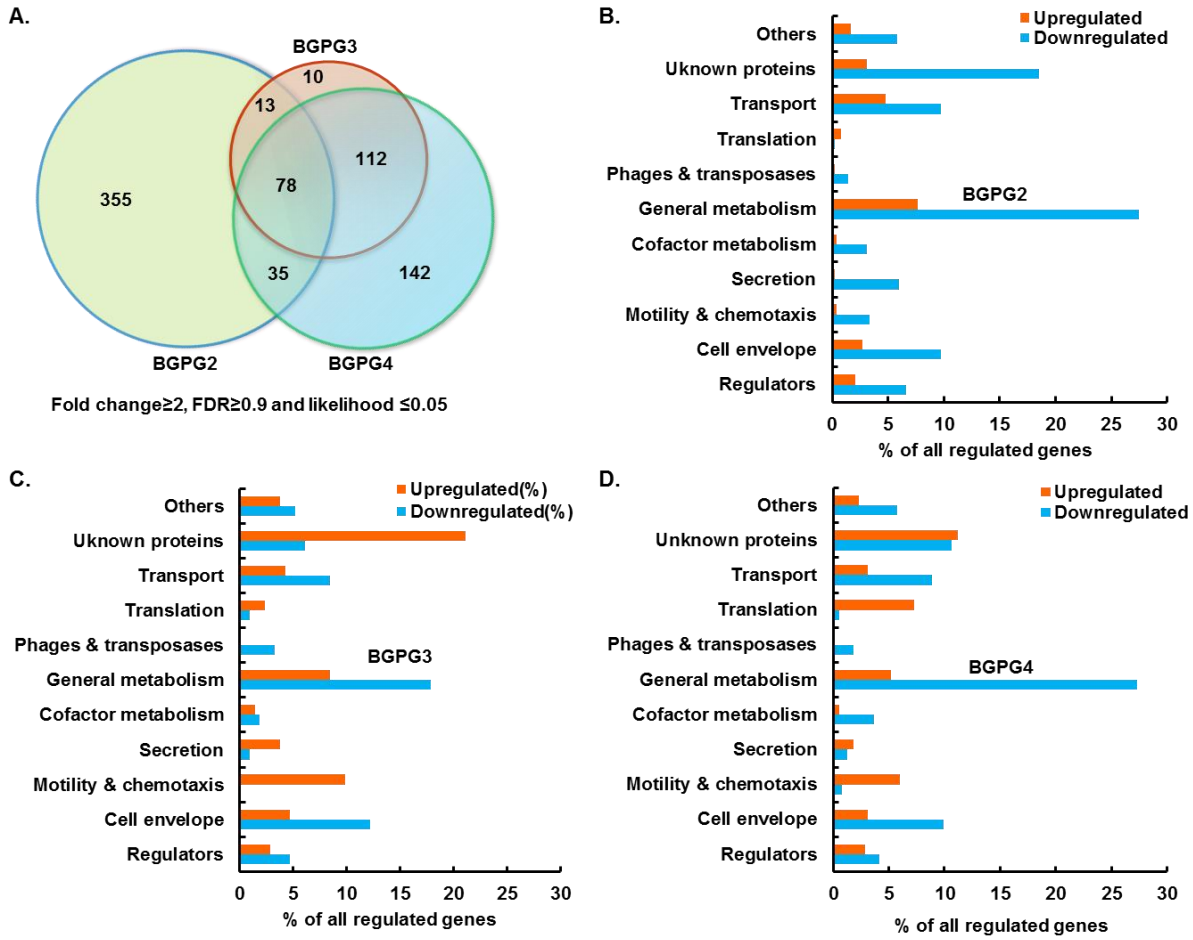


Figure 24. QS-regulated genes of BGPG2-4 vs. BGPG1. (A) Venn diagrams show the relationships of QS-regulated genes among BGPG2-4. Each circle shows the number of uniquely regulated genes in each BGPG1 mutant vs. BGPG1. The circles also show the number overlapping regulons under different mutants. **(B-D)** Function-based classification of QS-regulated genes in BGPG2-4. The catergories were based on the KEGG database (http://www.genome.jp/kegg/pathway.html).

Then these DEGs were classified into eleven functional categories based on the KEGG database (http://www.genome.jp/kegg/pathway.html) (Figure 24B-D). Results revealed that the regulated genes were mainly linked to general metabolism (Figure 25A cluster 11; Figure 25B cluster 2, 5 and 10) and hypothetical proteins (Figure 25A cluster 9; Figure 25B cluster 7 and 9; Table 18). A complete list of all QS-regulated genes is given in Table 18. The spectacular changes in gene expression of selected functional categories will be discussed in the following part.

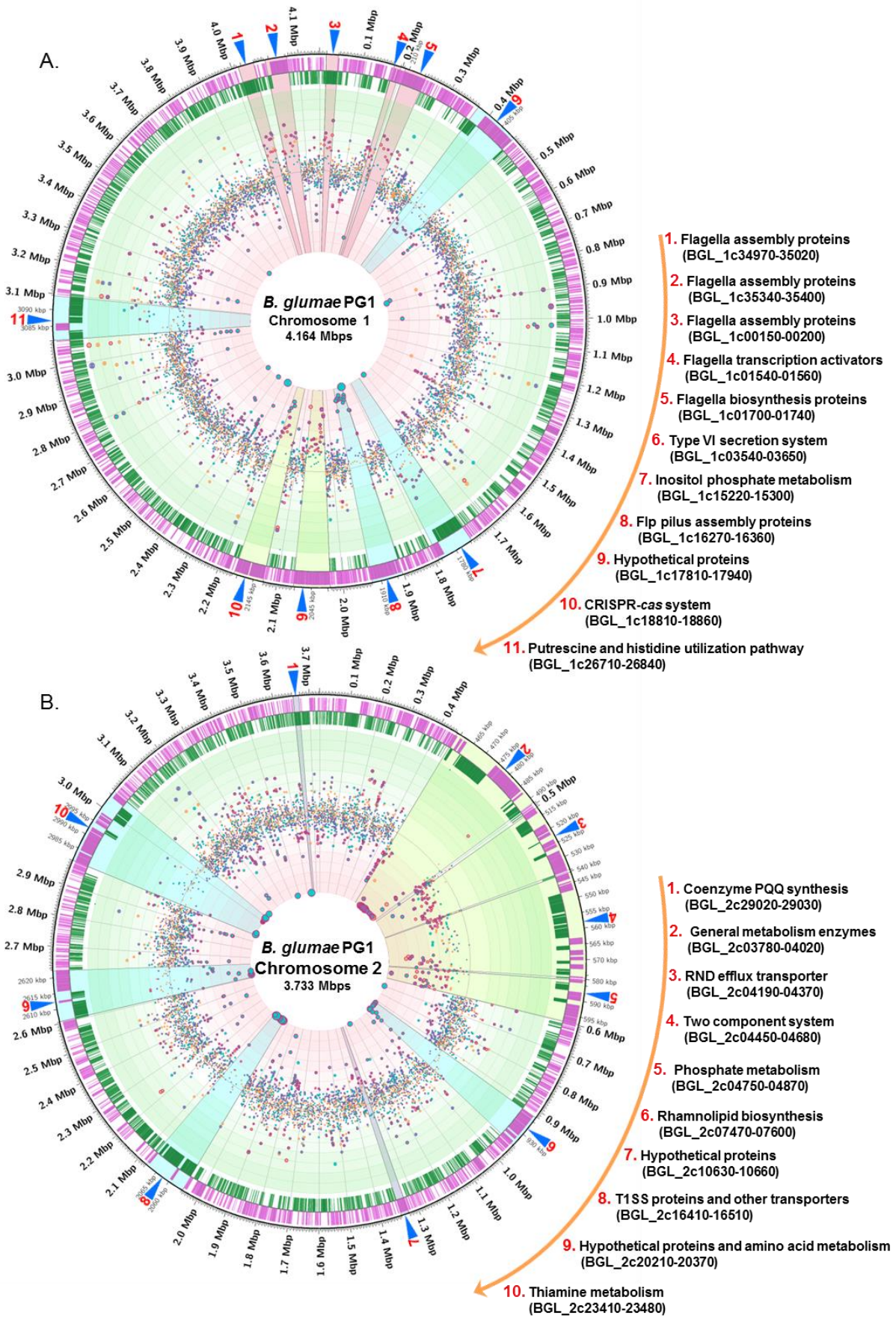


Figure 25. Circular transcriptome maps of BGPG2-4 vs. BGPG1 by the Circos software 0.64 (Krzywinski et al., 2009). Cyan dots, orange dots and deep purple dots represent genes from

BGPG2-4 strains, respectively. Dots with red violet circles represent QS-regulated genes. The cut-off was set to a fold-change of 2.0 with FDR ≤ 0.05 (dot size by values). Circles are described from the outside to the innermost circle: the first circle indicates the genome coordinates of BGPG1 in mega base pairs; the second and third circles indicate ORFs on the leading (purple) and the lagging (deep green) strands. The light green areas represent genes with log2 fold changes of 4; 3; 2; 1 (from outside to inside); the light red circles represent genes with log2 fold changes of -1; -2; -3; -4 (from outside to inside). Highlighted areas (labeled with numbers) are 5-fold magnified and show gene clusters which are QS-regulated in the mutant strains. Clusters showed in light cyan and light purple represent genes only QS-regulated in BGPG2 and BGPG4, respectively. Clusters showed in light pink represent genes QS-regulated in both BGPG3 and BGPG4. Clusters showed in pale green represent genes QS-regulated in all BGPG2, BGPG3 and BGPG4. **(A)** Circular map representing RNA-seq data from chromosome 1 of BGPG1. **(B)** Circular map representing RNA-seq data from chromosome 2 of BGPG1.

9.4. Validation of RNA-seq data by quantitative RT-PCR

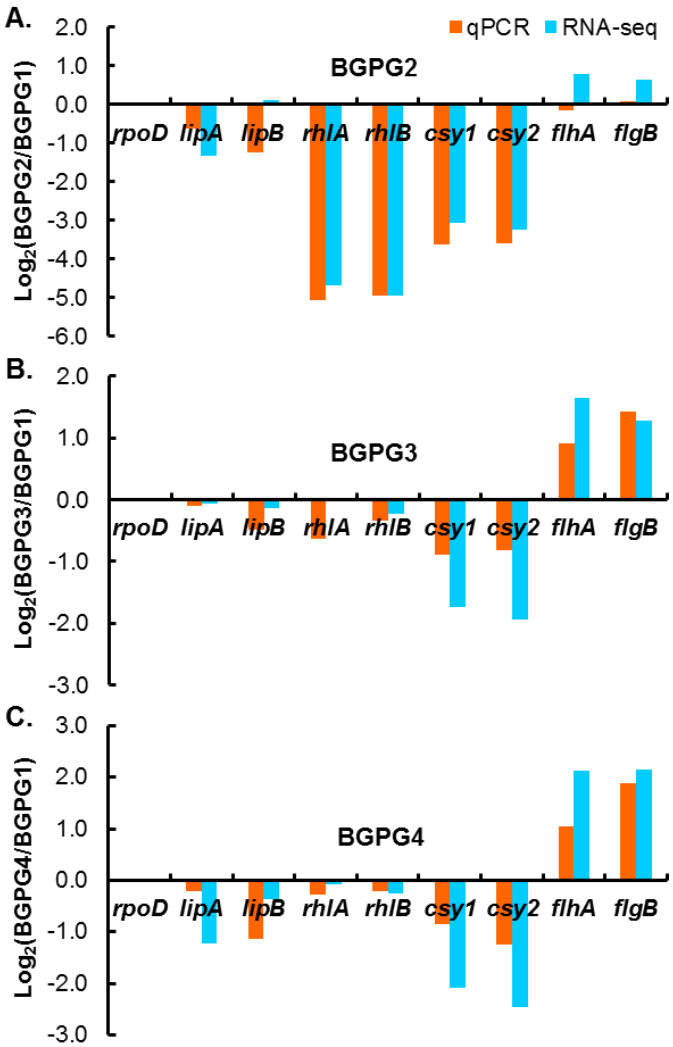


Figure 26. Validation of RNA-seq data by quantitative RT-PCR. (A) Fold change differences of BGPG2 to BGPG1 in the transition from exponential to stationary growth phase. **(B)** Fold change differences of BGPG3 to BGPG1 in the transition from exponential to stationary growth phase. **(C)** Fold

change differences of BGPG4 to BGPG1 in the transition from exponential to stationary growth phase. Each fold difference was calculated by qPCR using comparative quantification method, and log 2 ratio of obtained values was compared with log2 ratio of (BGPG2/BGPG1), (BGPG2/BGPG1) and (BGPG2/BGPG1) NPKM values. Orange bars indicate their fold change differences from qPCR; blue bars indicate their fold change differences from RNA-seq.

Eight of the QS-regulated genes were randomly selected to validate RNA-seq results using qPCR with specific primers (Table 12). As shown in Figure 26, the expression levels of selected genes by qPCR are in good accordance with results of RNA-seq, although there were minor differences in the altitude of fold change for a few genes. This could be due to different sensitivity of each technique. These data confirmed the reliability of results by RNA-seq.

IV. Discussion

1. BGPG1 harbors three AHL synthase genes

The BGPG1 genome has been fully sequenced recently and three AHL synthase homologue genes (*bgaI1-3*) were found (Knapp *et al.*, 2015; Voget *et al.*, 2015). So far, only one AHL synthase gene (*tofI*) was identified from each *B. glumae* isolate. It is interesting to test if all of these three AHL synthase homologue genes are functional in BGPG1 or not. To achieve this, firstly, each gene was cloned into *E. coli* strain. The AHL production was determined by AHL reporter bacteria *A. tumefaciens* NTL4 and *C. violaceum* CV026. Results have shown that AHL production of all three genes were positive for NTL4 (Figure 16B) and only AHL production of *bgaI1* could be detected by CV026 (Figure 16 E and F), suggesting AHL from *bgaI1* is C6 or C8-AHL, *bgaI2* and *bgaI3* encode for longer acyl chain length AHLs. Further studies with TLC overlay assay of cell-free supernatant extract from *bgaI1-3 E. coli* clones indicated that AHL of *bgaI1* is C8-oxo-AHL, AHL of *bgaI2* is C10-oxo-AHL and AHL of *bgaI3* could be C10-AHL or C12-oxo-AHL (Figure 16D). The parent strain BGPG1 reproducibly produced these spots on TLC plates (Figure 18D). Further, each of the Δ*bgaI* mutants produced one spot less than the parent strain and the individual AHL profiles of BGPG2-4 were different (Figure 18D). This observation confirmed above results and demonstrated that AHLs synthesized by each *bgaI* are different. Hence, BGPG1 harbors three AHL synthase genes *bgaI1-3* which is unique from other *B. glumae* strains that only contain one AHL synthase gene.

With this framework, the presence of multiple AHL synthase genes is a common feature within the genus *Burkholderia*, such as *btaIR1-3* in *B. thailandensis*, *bpsIR1-3* in *B. pseudomallei*, *cepIR* and *cciIR* in *B. cenocepacia*, *cepIR* and *bviIR* in *B.vietnamiensis* and so on (Majerczyk *et al.*, 2014b; Malott & Sokol, 2007; Malott *et al.*, 2005) (Table 2). In addition, many Gram-negative bacteria regulate their group behavior with multiple LuxIR homologous pairs and different AHL molecules (Jones *et al.*, 2002; Majerczyk *et al.*, 2014b). So far, the mechanism of how these multiple QS-systems work with each other is not well-studied. It has been shown that *P. aeruginosa* possesses three different QS systems: two *luxIR* homologue

QS systems (*lasIR* and *rhlIR*) and one *Pseudomonas* quinolone signal (PQS) QS system (Venturi, 2006). These systems form a hierarchy with the *lasIR* system at the top, positively regulating the other two QS systems. In addition, the PQS and *rhlIR* systems regulate each other mutually. In this work, transcriptome analyses results of BGPG1 and AHL synthase deletion mutants showed the deletion of *bgaI1* gene caused 5.9- and 10.5-fold down-regulation of the expression profile of *bgaIR2* and 2.9-fold down-regulation of the *bgaI3* (Figure 27B), suggesting *bgaI1* positively regulating *bgaI2* and *bgaI3*. The deletion of *bgaI2* had no obvious effects on expression of both *bgaIR1* and *bgaIR3*, and the deletion of *bgaI3* caused 4.3- and 5.7-fold down-regulation of *bgaIR2*, but had no obvious effect on *bgaIR1* (Figure 27B), indicating *bgaI3* positively regulating *bgaI2*. Collectively, these data raise the possibility that in BGPG1 three QS systems form a network with *bgaIR1* at the top hierarchy followed by *bgaIR3*, which could be overall similar to the complex hierarchical regulatory network observed in *P. aeruginosa* (Figure 27A).

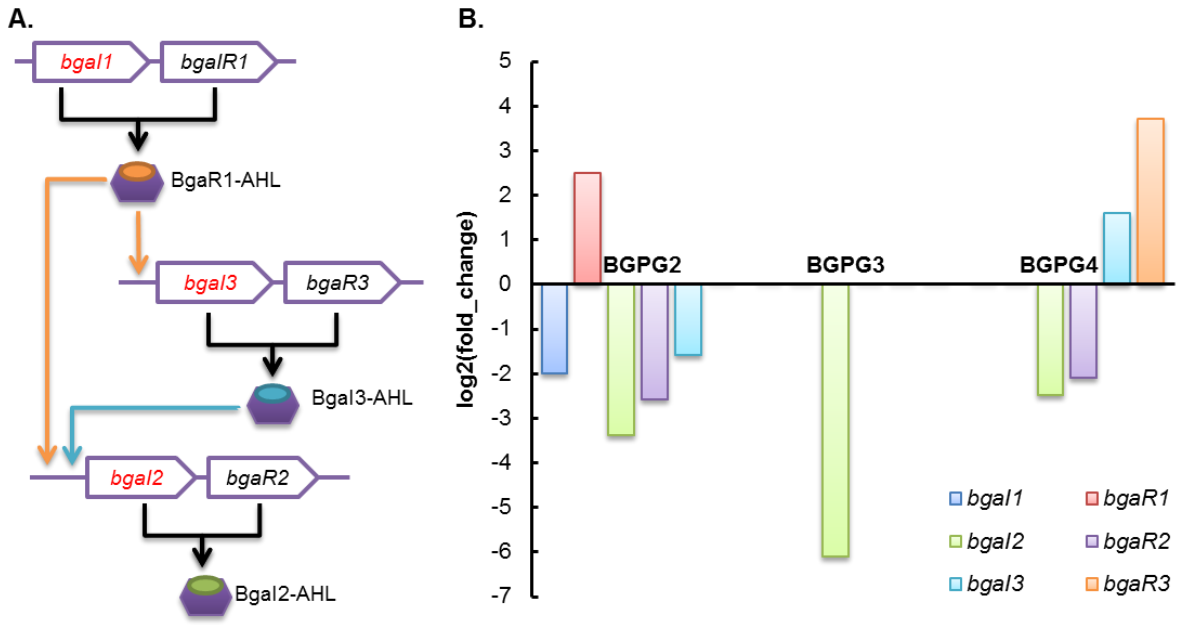


Figure 27. BGPG1 harbors a QS hierarchy. (A) QS systems in BGPG1 **(B)** Relative transcript levels of *bgaIR1-3* genes in BGPG2-4 vs. BGPG1.

2. Motility is regulated by QS systems in BGPG1

2.1. Motility phenotype analyses

2.1.1. Surface motility

Motility is one of the well-studied bacterial physiologies due to its essential roles in bacterial

lifestyle. Henrichsen *et al.* defined six different types of bacterial surface motility: swarming, swimming, gliding, twitching, darting and sliding, based on an investigation of hundreds of strains from 40 bacterial species (Henrichsen, 1972). Among them, swarming is the overall movement of bacteria and swimming is an individual endeavor, which are two best-studied surface motilities modulated by QS system in many Gram-negative bacteria, such as *S. meliloti*, *Y. enterocolitica*, *E. carotovora*, *B. cepacia* (Atkinson *et al.*, 2006; Chatterjee *et al.*, 2010; Hoang *et al.*, 2008; Huber *et al.*, 2001).

It was shown in this study BGPG1 strongly exhibited these two characteristic surface motility phenotypes, and deletion of each AHL synthase gene (*bgaI1-3*) greatly impaired both motilities (Figure 21A-B). Moreover, swarming motility was further confirmed by reintroducing relevant *bgaI* genes into mutant strains BGPG2-4 (Figure 21C). These observations are in line with data found in *P. aeruginosa*, in which all three QS systems (*las*, *rhI* and PQS) have been demonstrated to modulate surface motility (Glessner & Smith, 1999; Ha *et al.*, 2011; Köhler *et al.*, 2000; Lee & Zhang, 2014). A previous study has shown that a wild type *B. glumae* strain BGR1 produces polar flagella and contains a single LuxIR type QS system, TofIR (Kim *et al.*, 2007). The deletion of AHL synthase *tofI* gene resulted in a strong impairment of swarming and swimming motilities, which could be restored by the reintroduction of the *tofI* gene into the mutant strain (Jang *et al.*, 2014; Kim *et al.*, 2007). It has been demonstrated that motility is essential for BGR1 to infect rice plants and non-motile mutants are attenuated in their virulence. Although BGR1 only contains one QS system, data from this strain are in line with the observation of motility in BGPG1 and three *bgaI* genes deletion mutants, suggesting that surface motility of BGPG1 could be regulated by all three AHL-synthase genes and also implying the connection of the virulence and QS systems of BGPG1.

2.1.2. Liquid motility

Sedimentation assays were performed to identify the liquid motility of the parent strain BGPG1 and mutant strains BGPG2-4 in TY medium. Results (Figure 21D) showed that both BGPG1 and BGPG2 can settle down in TY medium and form loose pellets at the bottom of

glass tubes after few hours standing at room temperature. However, BGPG3 and BGPG4 were not able to form sediment within 42 h (Figure 21D). Furthermore, the phenotypes of BGPG3 and BGPG4 can be partially restored by using complemented strains BGPG3c and BGPG4c (Figure 21D). These results suggested that both *bgaI2* and *bgaI3* other than *bgaI1* play roles on regulating sedimentation in BGPG1. Similar sedimentation phenotypes were observed by Krysciak and colleagues in *Sinorhizobium fredii* NGR234 (Krysciak *et al.*, 2014) and by Hubert and associates in *Legionella pneumophila* (Kessler *et al.*, 2013; Tiaden *et al.*, 2010b). Using the electron microscopy to analyze the ultrastructure of *L. pneumophila* in sediments and suspension, they further proved that the formation of an extracellular "matrix" by production of extracellular filaments could be a possible reason of impaired sedimentation of *Legionella pneumophila* after deletion of QS molecular signal (LAI-1) synthase gene *lqsA* (Kessler *et al.*, 2013; Tiaden *et al.*, 2010b). In this study, results of RNA-seq analyses showed two filament-associated clusters (Figure 25A, cluster 2 and 5) including flagellin-specifics chaperone gene *fliS* (Galeva *et al.*, 2014), flagellar export protein gene *fliJ* (Ibuki *et al.*, 2013; Minamino *et al.*, 2000), flagella RNA polymerase gene *fliA* (Starnbach & Lory, 1992), flagellar biosynthesis protein gene *flhA* (Ibuki *et al.*, 2013)(Kinoshita *et al.*, 2013), were 2-4.4-fold up-regulated in BGPG3 and BGPG4 (Table 18). These results suggested sedimentation in BGPG1 could be regulated by BgaI 2-3-QS systems in a similar mechanism as in *Legionella pneumophila.*

2.2. BGPG1 QS systems regulate motility through modulating flagella genes.

Most motile bacteria move by using flagella which containing three major domains: an ion driven motor, a hook and the filament. Bacterial flagellum synthesis is a complex process involving in more than 50 genes, including structure genes and regulation genes, which are highly conserved in bacteria (Aldridge & Hughes, 2002; Chevance & Hughes, 2008; McCarter, 2006). Flagellated bacteria regulate the expression of structural genes through a transcriptional hierarchy (Chevance & Hughes, 2008). The first genes to be transcribed, designated as early genes, encode the master regulators including FlhDC, FleQ, and FlrA. These master regulators initiate the transcriptional hierarchy and promote transcription of structure and regulation genes, designated as middle genes, which activate expression of late

genes (Smith and Hoover 2009).

Many bacteria modulate motility by flagella in a quorum sensing dependent manner. For instance, Yang and Defoirdt demonstrated that the deletion of each of 3 different autoinducer synthase genes in *Vibrio harveyi* significantly inhibited expression of flagella genes, resulting in impaired motility (Yang & Defoirdt, 2014). In Helicobacter pylori, the QS singal molecule autoinducer-2 was reported to function as a secreted signaling molecule upstream of FlhA and plays a critical role in global regulation of flagella gene transcription to regulate motility (Rader *et al.*, 2007). BGR1, another member of *Burkholderia glumae* was reported to employ QsmR as a master regulator to initiate transcription of the early structural genes in a QS-dependent manner (Kim *et al.*, 2007).

In BGPG1, 68 genes, grouped into 8 clusters on chromosome 1, are involved in flagella biosynthesis and responsible for encoding the polar flagellum. Among them, 23 genes have two replications. It has been discussed above that the deletion of each AHL synthase gene largely impaired surface motility of BGPG1 (Figure 21A-B) and the flagella could be the most important reason. The attempt to observe the morphology difference between the parent strain BGPG1 and deletion mutants BGPG2-4 by TEM was failed. However, RNA-seq data in this study indicated that the majority of flagella-associated genes, including the flagellar protein export genes (*fliQ,flip, flhB, flhA, flhF* and *fliH*), the flagellar C ring genes (*fliM* and *fliG*), the flagella motor gene (*flhG*), the flagella chaperon genes (*fliS* and *fliJ*), the flagellar rod, hook and filament genes (*fliL, flgG, flgE, flgB* and *flgD*), the flagellar M, S, P and L rings genes (*fliF*) and the flagellar regulation genes (*flhD, flhC* and *fliA*) (Figure 25A, clusters 1-5), were up-regulated 2.0-5.3-fold in two ∆*bgaI* mutants BGPG3 and BGPG 4 compared to the parent strain. These findings of the higher transcription level of the flagella genes are in line with reports from *S. fredii* NGR234 in the background of two AHL mutants (Krysciak *et al.*, 2014) and it is consistent with increased transcription of flagella genes in BGR1 (Kim *et al.*, 2013) and *B. gladioli's tofI* mutant (Kim *et al.*, 2014).

In different species, the flagella master regulators (FlhDC) were modulated by different factors such as heat shock response network proteins DnaK, DnaJ and GrpE (Li *et al.*, 1993; Shi *et*

al., 1993), histone-like nucleoid-structuring (H-NS) protein (Soutourina & Bertin, 2003), quorum sensing master regulator QsmR (Kim *et al.*, 2007) and quorum sensing *Escherichia coli* regulators QseBC (Sperandio *et al.*, 2002). DnaK, DnaJ and GrpE are required for the flagella biosynthesis in *E. coli* through modulating transcription of the *flhDC* operon and *fliA* operon (Shi *et al.*, 1992). In BGPG1 these three genes are located in a single cluster, RNA-seq data indicated that the deletion of *bgaI3* gene resulted in a 2.1-fold down-regulation of DnaK (Table 18). The H-NS protein was shown to act as a positive regulator of genes involved in the biogenesis of flagella (Bertin *et al.*, 1994), which was 8.7-fold down-regulated in *bgaI1* deletion mutant compare to wide type. QsmR was found to promote transcription of FlhDC and flagella biosynthesis in BGR1(Jang *et al.*, 2014; Kim *et al.*, 2007). Although BGPG1 encodes one orthologous gene of *qsmR* (BGL_1c10570) in chromosome 1 (95% in amino acid sequence identity), RNA-seq data showed that the expression of *qsmR* was not affected by deletion of each *bgaI* gene in BGPG1, indicating that BGPG1 QS systems do not activate the expression of *flhDC* genes by the activation of *qsmR* gene. Although no direct evidence has shown the involvement in motility of the *qseBC* genes for *B. glumae*, it is observed that *qseBC* genes were 2.1-2.5-fold down-regulated in the three mutants compared to parent strain (Figure 25B, cluster 4). These results suggested that the different regulators rather than structural features were responsible for the observed flagella-dependent surface motility phenotypes. Proteins DnaK, H-NS and QseBC were promising regulator candidates in BGPG1.

2.3. BGPG1 QS systems regulate motility through modulating Type IV pilus genes

Besides flagella, type IV pilus (Flp pili) is another factor considered to influence bacteria surface motility in a QS-dependent manner. Type IV pilus has been identified in many bacteria and archaea (Kachlany *et al.*, 2001; Planet *et al.*, 2001; Tomich *et al.*, 2007). It was reported that they are involved in bacteria surface attachment (Wall & Kaiser, 1999), protein secretion (Hager *et al.*, 2006), DNA uptake (Chen & Dubnau, 2004) and pathogenic interactions with eukaryotic hosts (Craig *et al.*, 2004). The bacteria movement mediated by type IV pilus over surfaces without the use of flagella, known as twitching motility, was used by many bacteria, such as *M. xanthus*, *P. aeruginosa* and *N. gonorrhoeae* (Kohler *et al.*, 2000; Oomey, 1998;

Sun *et al.*, 2000). The tad (tight adherence) macromolecular transport system, which represents an ancient subtype of the type II secretion system (T2SS), is necessary for built-up of pilus (Tomich *et al.*, 2006, 2007).

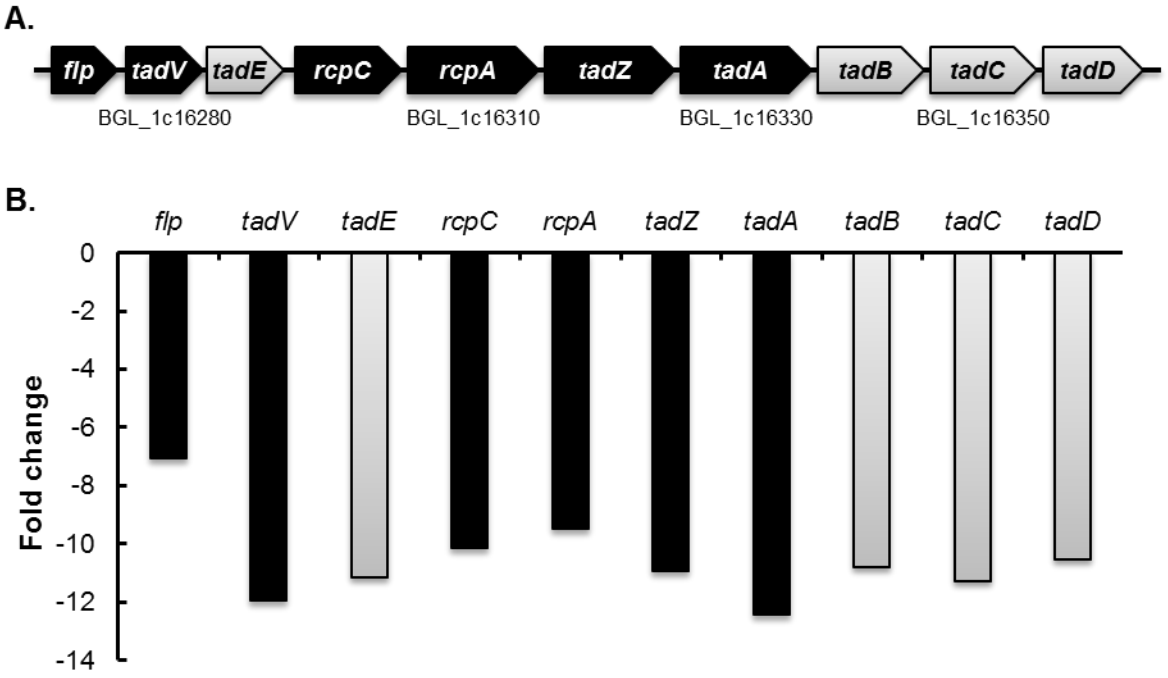


Figure 28. Flp pilus biosynthesis is QS-regulated in BGPG1. **(A)** Genomic organization of tad locus in BGPG1. **(B)** Relative transcript levels of the tad cluster genes in BGPG2 vs. BGPG1.

BGPG1 has a single cluster encoding for type IV pilus genes located on chromosome 1 (Figure 28A). In three *bgaI*-deletion mutants BGPG2-4 the flp pilus gene cluster was down-regulated, especially in BGPG2 with a more than 10-fold down-regulation compared to the parent strain (Figure 25A and cluster 8; Figure 28 B; Table 18). These data are in line with results found by Glessner and colleagues in *P. aeruginosa*, in which they demonstrated *las* system is necessary for effective twitching motility and *rhl* system can influence export and surface assembly of flp pili (Williams *et al.*, 2000).

Although the deletion mutants in type IV pilus genes have not been constructed and tested in this study, a paper published in 2011 by Taguchi and Ichinose demonstrated that mutations of type IV pilus genes resulted in strongly impaired swarming and swimming motilities of *P. syringae* pv. *tabaci* 6605 (Taguchi & Ichinose, 2011). Within this framework, it is reasonable to hypothesize that type IV pilus regulates surface motility on a QS-dependent manner in BGPG1 as well.

2.4. BGPG1 QS systems regulate motility through modulating rhamnolipid production

Rhamnolipid is produced from its precursor 3-(3-hydroxyalkanoyloxy) alcanoic acid (HAA) in *P. aeruginosa*. The synthesis of HAA is catalyzed by RhlA. Then two special rhamnosyltransferases RhlB and RhlC catalyze the sequential rhamnosyl transfer reactions from HAA over mono- toward di-rhamnolipids, using dTDP-L-rhamnose as rhamnosyl donors (Caiazza *et al.*, 2005; Deziel, 2003; Ochsner *et al.*, 1995; Rahim *et al.*, 2001). The dTDP-L-rhamnose is converted from α-D-glucose-6-phosphate under the control of phosphoglucomutase AlgC (Olvera *et al.*, 1999) and four enzymes: RmlB, RmlD, RmlA and RmlC (Aguirre-Ramírez *et al.*, 2012).

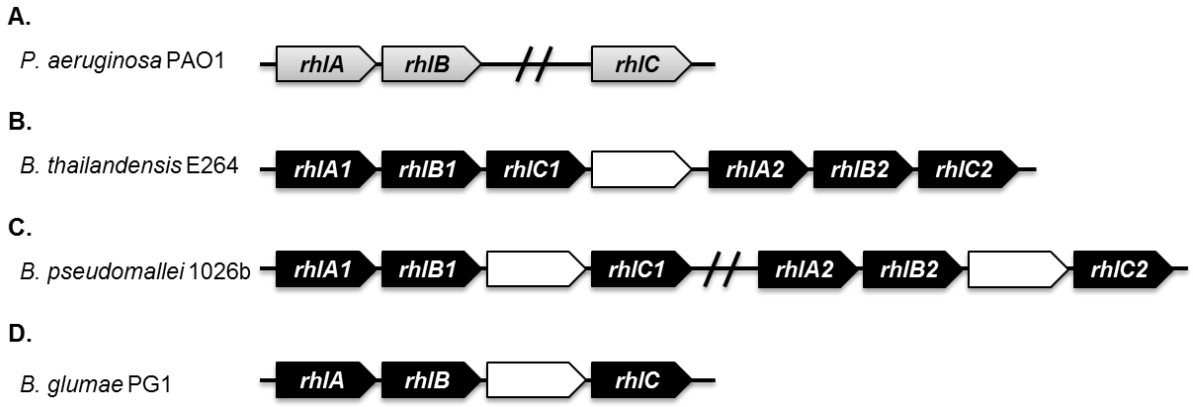


Figure 29. Physical maps of *rhlABC* genes in BGPG1 and the selected strains. (A) Physical map of *rhlABC* genes in *P. aeruginosa* PAO1. **(B)** Physical map of *rhlABC* genes in *B. thailandensis* E264. **(C)** Physical map of *rhlABC* genes in *B. pseudomallei* 1026b. **(D)** Physical map of *rhlABC* genes in BGPG1.

Rhamnolipid was firstly found to be produced by strain *P. aeruginosa*, which has a lot similarity with the genus *Burkholderia* (Yabuuchi *et al.*, 1992). It is known that some *Burkholderia* species also can produce rhamnolipid, such as *B. pseudomallei*, *B. thailandensis,* and *B. kururiensis* (Dubeau *et al.*, 2009; Nimtz *et al.*, 1998; Tavares *et al.*, 2013). For *B. glumae,* one strain AU6208 has reported to be able to produce considerable amounts of rhamnolipids with longer side chains (Costa *et al.*, 2011).

Similar as *P. aeruginosa*, BGPG1 contains all components that are necessary for producing rhamnolipid including *RhlA, RhlB, RhlC, AlgC, RmlA, RmlC* and *RmlD*. Among them, *RhlA,*

RhlB and *RhlC* are located in the same operon which is contrast to *P. aeruginosa*, in which *rhlC* is separate from *rhlAB* (Figure 29A). BGPG1 only contains one copy of *rhlABC* cluster that is different from other *Burkerderia* species, such as *B. thailandensi* and *B. pseudomallei* which harbor two copies (Figure 29B-D).

It is known in *P. aeruginosa* the production of rhamnolipid is regulated in a QS-dependent manner. The *rhl* QS system can directly reregulate *rhlAB* expression through modulating *rhlA* promoter (Medina *et al.*, 2003). PQS and *las* QS systems indirectly regulate expression of *rhlAB* by control of *rhlR* transcripition (Déziel *et al.*, 2005; Diggle *et al.*, 2003; Jensen *et al.*, 2006; Medina *et al.*, 2003; Pearson *et al.*, 1997). Furthermore, rhamnolipids have been reported to serves as a surface wetting agent and chemotaxis stimulus and has an effect on surface motility in *P. aeruginosa* (Caiazza *et al.*, 2005).

Table 15. Relative transcript levels of rhamnolipid biosynthesis genes in BGPG2-4 vs. BGPG1.

Locus tag	Gene	Fold change		
		BGPG2	BGPG3	BGPG4
BGL_1c07250	*rmlA*	1.3-	1.1-	1.2-
BGL_1c07260	*rmlC*	1.1-	-	1.1-
BGL_1c07270	*rmlD*	1.1-	1.1-	1.4-
BGL_1c07370	*algC*	1.3-	1.2-	1.3-
BGL_2c07470	*rhlA*	26.0-	-	1.1-
BGL_2c07480	*rhlB*	30.7-	1.2-	1.2-
BGL_2c07500	*rhlC*	29.9-	1.1+	1.3-

Transcriptome data in this study showed that the *rhlABC* transcription levels were decreased after deletion of each AHL synthase gene, with exceptions of *rhlA* and *rhlC* in mutant strain BGPG3 (Table 15). Notably, the expression levels of *rhlABC* were decreased greater than 26-30-fold after deletion of *bgaI1* gene, indicating the dominant effects of *bgaI1* on regulating production of rhamnolipid. Furthermore, RNA-Seq results showed that deletion of each *bgaI* gene results in the down-regulation of *algC* and *rmlACD* (Table 15). These data highly

suggested that the production of rhamnolipid is QS-regulated, especially *bgaI1*-regulated, in BGPG1. Due to the absence of deletion mutants of rhamnolipid genes, direct evidence of effects of rhamnolipid on bacteria motility cannot be obtained. However, Danielle and colleagues observed that swarming motility is completely abolished in double Δ*rhlA* mutant of *B. thailandensis* (Dubeau *et al.*, 2009). Therefore, it is reasonable to suggest the positive roles of rhamnolipid on bacteria motility in BGPG1.

2.5. BGPG1 QS systems regulate motility through modulating metabolic activity

Table 16. QS-regulated metabolic genes involved in the regulation of motility in BGPG1.

Locus_tag		Function	Fold change		
BGPG1	**PAO1**		**BGPG2**	**BGPG3**	**BGPG4**
BGL_1c08720	PA4282	DNA polymerase III	2.7-	1.3-	1.3-
BGL_1c16420	PA0887	AMP-dependent synthase and ligase	1.7-	1.6-	4.0-
BGL_1c24890	PA2634	Long-chain acyl-CoA synthase FadD	1.6-	1.8-	2.0-
BGL_2c03920	PA0186	ABC transporter family protein	32.5-	18.8-	22.5-
BGL_2c04750	PA2332	transcriptional regulator	3.0-	2.1-	2.3-
BGL_2c04810	PA0186	2-aminoethylphosphonate pyruvate transaminase Phnw	8.4-	6.2-	14.3-
BGL_2c04850	PA0186	2-aminoethylphosphonate transport permease PhnV	3.2-	1.8-	2.4-

Metabolic activities is another factor which is supposed to influence bacterial motility in a QS-dependent manner (Kim *et al.*, 2013). In *B. glumae* BGR1, *astC* and *fadE* are involved in arginine degradation II and fatty acid β–oxidation I, respectively. And the deletion of each gene resulted in a strong reduction of swarming motility under the regulation of QS process (Goo *et al.*, 2012; Pai *et al.*, 2012). In *P. aeruginosa* PAO1, using Mini-Tn5 mutant library, Overhage and colleagues characterized a set of genes involved in swarming motility, most of which are involved in metabolism processes (Overhage *et al.*, 2007). A comparison analysis of these genes in PAO1 and QS-regulated genes in BGPG1 revealed that 7 genes are highly

homologous in two strains in amino acids level and are significantly down-regulated in all *bgaI* deletion mutants of BGPG1 (Table 16). Notably, genes *phnW* and *phnV* are located at the *phnWSTUVA* operon, which encode a series of enzymes required for phosphate metabolism, suggesting phosphate metabolism pathway could be involved in regulation of motility in a QS-dependent manner in BGPG1.

Altogether, these findings suggested that BGPG1 motility is modulated in QS-dependent manner through regulating different factors, such as rhamnolipids, metabolic activities, flagella and flp pili. While it is reasonable to speculate that there are still some unknown pathways or activities controlled by QS systems to modulate bacterial motility.

3. Lipolytic activity is regulated by QS systems in BGPG1

Lipolytic activity refers to the ability of lipids conversion by lipolytic enzymes. Lipolytic enzymes belong to the class of carboxylic ester hydrolases that catalyze both the hydrolysis and synthesis of ester bonds, and include esterases, commonly called carboxyesterases (EC. 3.1.1.1) and lipases (triacylglycerol hydrolases, E.C. 3.1.1.3). Based on the conserved amino acid sequence homology and biological properties, bacterial lipolytic enzymes can be classified into 8 families, which share a characteristic hydrolase fold in the three-dimensional structure (Arpigny & Jaeger, 1999). Quorum sensing was reported to plays a major role in bacterial extracellular lipolytic activity. Studies performed by Chrestensen and cooperators indicated that AHL-based QS system in *Serratia proteamaculans* strain B5a has an effect on the production of lipolytic activity, which could result in the spoilage of milk (Christensen *et al.*, 2003). In this study, the lipolytic activity of the parent strain BGPG1 and three mutant strains BGPG2-4 was detected on TBT plates by measuring clearing zones around colonies. Compared to parent strain BGPG1, the size of clearing zones was in general 2.5-fold smaller in the mutant strains BGPG2-4 (Figure 20A-B). Lipolytic activity was also quantitatively determined in liquid using *p*NP-octanoate as a substrate. Each mutant showed strongly reduced lipolytic activity which well confirmed the above results (Figure 20C). These findings indicated that all *bgaI*-QS systems have positive roles in the lipolytic activity of BGPG1.

Lipase is an important lipolytic enzyme with great industrial potential and produced by a large

variety of living organisms. It can be obtained from different sources such as microorganisms, animals and plants. In many bacteria, the lipase production is controlled by QS systems. One of the well-studied examples is the clinical isolate of *B. glumae* strain AU6208. The AHL synthase deletion mutant as well as the lipase *lipA* deletion mutant like *B. glumae* ATCC 33617, which has an inactive QS sensor/regulator TofR, were not able to produce LipA (Devescovi *et al.*, 2007). It is also demonstrated that introducing the *tofR* gene of *B. glumae* AU6208 in strain ATCC 33617 restored its ability to produce lipase LipA (Devescovi *et al.*, 2007), highlighting the importance of QS in regulation lipase production and activity. The study about another *Burkholderia* strain, *B. cenocepacia*, showed the deletion of AHL synthase genes resulted in 40% to 50% less lipase activity than the wild type strain at stationary phase (Lewenza *et al.*, 1999). Moreover, even in a non-pathogenic species *B. thailandensis*, the lipase activity and production were found to be modulated by three *luxIR*-type QS systems (Ulrich *et al.*, 2004).

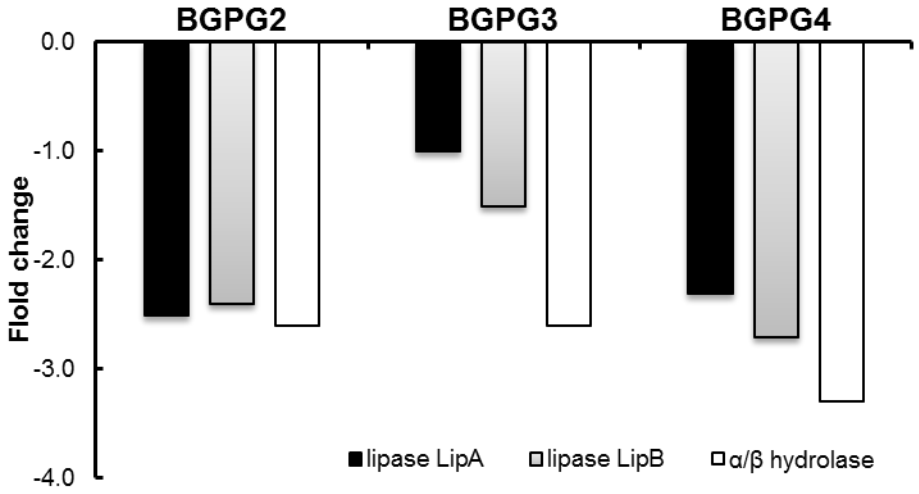


Figure 30. Relative transcript levels of lipase *lipAB* genes and a putative liplytic gene (BGL_2c04340).

In this work, RNA-seq data showed that the expression of lipase *lipA* (BGL_2c18660) was down-regulated 2.2- and 2.5-fold in BGPG2 and BGPG4 compared to BGPG1 (Figure 30 and Table 18). Interestingly, none of the genes associated with general secretion system (Sec secretion system) involved in transport of lipases (El Khattabi *et al.*, 2000) was found to be differentially regulated in the three mutants. This suggested that QS systems regulate lipase

activity through control expression of *lipA* rather than silencing its transport pathway.These observations are in line with data obtained for other *Burkholderia* strains AU6208 and ATCC 33617 (Devescovi *et al.*, 2007), especially for *B. thailandensis* (Ulrich *et al.*, 2004). Considering the fact that *B. thailandensis* also contains three QS system like BGPG1, more similarities between these two strains are expected. In addition, a putative alpha/beta hydrolase gene (BGL_2c04340) showed a 2.6-3.3-fold decreased transcription levels in all three ∆*bgaI* mutants (Figure 30). Given that *lipA* was not down-regulated in BGPG3, it was speculated that the alpha/beta hydrolase also contributed to lypolytic activity.

4. Colony variation and plant virulence are regulated by QS systems in BGPG1

Bacteria pathogenicity is correlative with their colony morphology which has been shown in *R.solanacearum* (Kelman, 1954). With the help of tetrazolium chloride (TZC) in solid media, the colony shape and color can be changed due to the formation of extracellular or capsular polysaccharides which are directly correlated with virulence (Kelman, 1954). As shown in Figure 22A, colonies of BGPG1 on TZC medium were irregularly-round, weakly undulate margin, fluidal, raised, light red and large in size, suggesting they are highly pathogenic. In contrast, colonies of BGPG2-4 were regularly-round, entire margin, deep red and pulvinate, implying they have weaker pathogenicity than wide type strain BGPG1. These results were consistent with observations obtained in *B. glumae* MAFF 302748 by Kato and colleagues. They demonstrated the strong connection between virulence of *B. glumae* MAFF 302748 and its colony morphology by infection of several plants (Kato *et al.*, 2013).

Since this test is well known to differentiate avirulent and virulent strains, it was speculated that all *bgaI*-deletions would affect the virulence activity of BGPG1. Therefore, onion maceration assay, which was previously used to determine the virulence of *B. glumae* strains (Chen *et al.*, 2012; Karki *et al.*, 2012), was set up to test pathogenicity of wild-type BGPG1 as well as the mutant strains on detached onion bulb scales. As expected, each of the mutant strains appeared to be attenuated in its capability to macerate onion tissues (Figure 22F). Specifically, BGPG1 showed a clear macerated tissue around the wound whereas BGPG3 showed a strongly reduced maceration and BGPG2 and BGPG4 showed no maceration at all.

Karki and cooperators have done one onion maceration assay with 11 naturally avirulent and 9 virulent isolates of *B. glumae* native from the southern United States and found that all of the 9 virulent isolates but none of the avirulent strains caused maceration of the onion bulb scale tissue (Karki *et al.*, 2012). Data obtained in this study together with published results suggested that BGPG1 could be a moderately virulent strain. Moreover, Chen and colleagues demonstrated that the deletion of *tofI* and *tofR* in *B. glumae* strain 336gr-1 make it lost the ability of maceration, indicating the importance of QS system in this phenotype (Chen *et al.*, 2012). Although there is no direct evidence of the connection between lipolytic activity and onion maceration, a number of bacterial secreted lipases or esterases have been reported to be involved in the penetration of plant barriers like waxes and cuticle (Aparna *et al.*, 2009; Feng *et al.*, 2005; Rajeshwari *et al.*, 2005; Voigt *et al.*, 2005). Devescovi and colleagues found that lipase activity is essential for rice pathogenicity of *B. glumae* strains AU6208 and ATCC 33617 (Devescovi *et al.*, 2007). Both AHL synthase deletion mutant and lipase *lipA* deletion mutant were no longer pathogenic to rice. They also proposed that maceration of plant tissues by *B. glumae* strains could be caused by the cooperation of lipases with other enzymes, which promotes the degradation of xylan and waxes. Interestingly, in this study it was shown that the deletion of *bgaI* genes largely reduced the lipolytic activity and transcriptional levels of the lipase *lipAB* genes and a putative alpha/beta hydrolase gene (BGL_2c04340) (Figure 30). All these data suggest that the encoded lipase could be a promising candidate for being responsible for the observed phenotype of onion maceration.

To further investigate the virulence of *B. glumae* strains against rice, the germination rate was monitored as well as the length of rice seedlings. All deletion mutants showed significantly reduced pathogenicity on rice seedlings and rice seed germination rate (Figure 22B-D). BgaI1 has a 95% identity with TofI which can positively regulate production of toxoflavin. As it is known toxoflavin is the main cause of panicle blight in *B. glumae*, it was expected that BgaI1 play the same role as TofI on panicle blight in rice. Surprisingly, neither the production of toxoflavin nor the panicle blight by BGPG1 and all mutant strains was observed. The sequence analysis revealed that BGPG1 only contains 3 homologue components of toxoflavin circuit genes and they are located separately in different chromosomes. However, the other *B.*

glumae stain BGR1, which can produce functional toxoflavin and cause rice panicle blight, contains 11 components of toxoflavin circuit genes and are located in the same cluster (Figure 31). Study by Chen and colleague showed that a toxoflavin-deficient strain is almost avirulent and the disruption of the toxoflavin biosynthesis pathway causes significantly less rice panicle blight (Chen et al. 2012). Therefore, it is likely that BGPG1 is not able to produce functional toxoflavin due to lack of most of toxoflavin circuit genes.

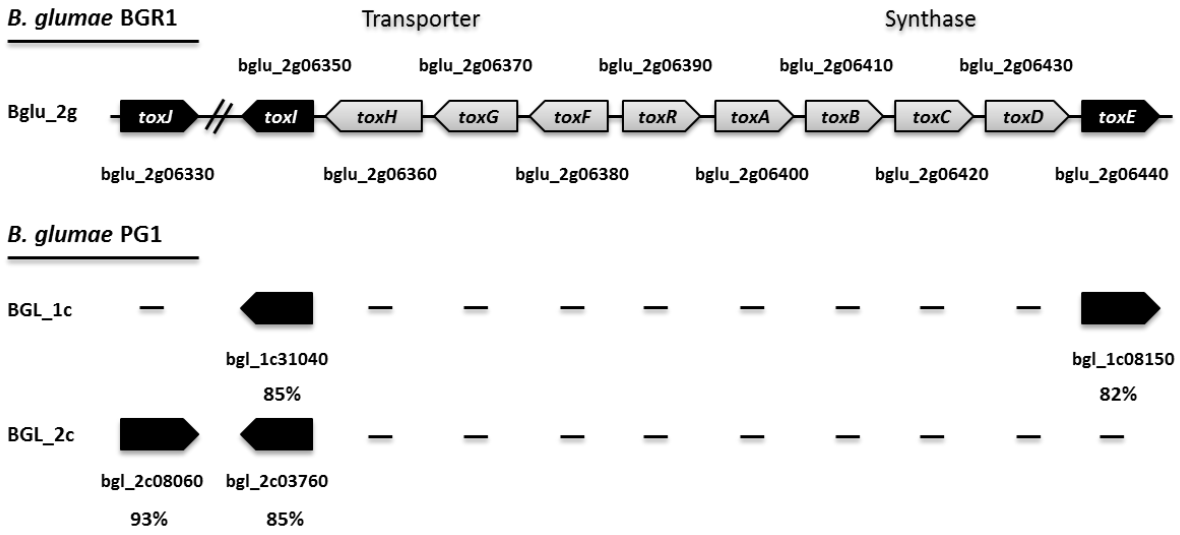


Figure 31. Comparison of the toxoflavin biosynthesis and tranporter gene clusters in BGPG1 and BGR1.

Since deletions of *bgaI1-3* had strong effects on pathogenicity, these findings suggest a more complex QS-dependent regulatory network in BGPG1 compared to BGR1 and other currently studied *B. glumae* isolates. Considering the fact that BGPG1 does not produce toxoflavin, it is possible that the lipolytic activity instead of toxoflavin is one of the virulence factors on rice that is modulated by these three QS systems in BGPG1.

5. Analyses of gene regulation by QS systems in BGPG1

The genome of *B. glumae* PG1 consists of 2 chromosomes encoded for 6,502 genes. Chromosome 1 encodes for 3,562 genes and has a size of 4.146 Mbp. Chromosome 2 encodes for 2,940 genes and has a size of 3.733 Mbp.

Transcriptomic analysis of three *bgaI* mutants BGPG2-4 in comparison with parent strain

BGPG1 revealed that a total of 745 genes were differentially regulated in a QS-dependent manner, making up 11.5% of all identified genes. Of these, 481 genes were differentially regulated by *bgaI1*-QS system, representing approximately 6.5% of all predicted genes (Figure 24A). 213 genes were significantly regulated by *bgaI2*-QS system, comprising 2.9% of all predicted genes (Figure 24A). 367 genes (4.9 % of all predicted genes) were significantly altered in the *bgaI3*-deletion strain BGPG4 (Figure 24A). Furthermore, a subset of 78 genes was differentially co-regulated in all of three *bgaI*-deletion mutant strains in comparison to the parent strain BGPG1. In addition, 355 genes were specifically regulated in *bgaI1*-deletion strain BGPG2, 10 genes in *bgaI2*-deletion strain BGPG3, and 142 genes in *bgaI3*-deletion strain BGPG4 (Figure 24A).

In BGPG1 *bgaI1 and bgaI2* QS systems are found on the chromosome 2 while the *bgaI3* is encoded on the larger chromosome 1 which harbors the housekeeping genes. This observation differs from the situation in *B. thailandensis*, in which all of three QS systems were found on chromosome 2. In BGPG1, the majority of QS-regulated genes (405 genes) are located on the chromosome 2, and 340 genes are located on chromosome 1. These findings are in line with data obtained by Greenburg and cooperators in *B. thailandensis* in which a total of 56.8% of 542 QS-regulated genes in the T phase only locate on chromosome 2 (Majerczyk *et al.*, 2014a). Furthermore, research performed by Vaughn and colleagues indicated that chromosome 2 in bacteria serve as an accessory genome for specific niches or conditions and the chromosome 1 encodes for more essential functions which ensures the bacterial prolonged maintenance (Cooper *et al.*, 2010). Thus, it at least suggested that *bgaIR3* system might be indispensable for BGPG1 and *bgaI1-2* QS systems play important roles on adapting bacteria to different environments.

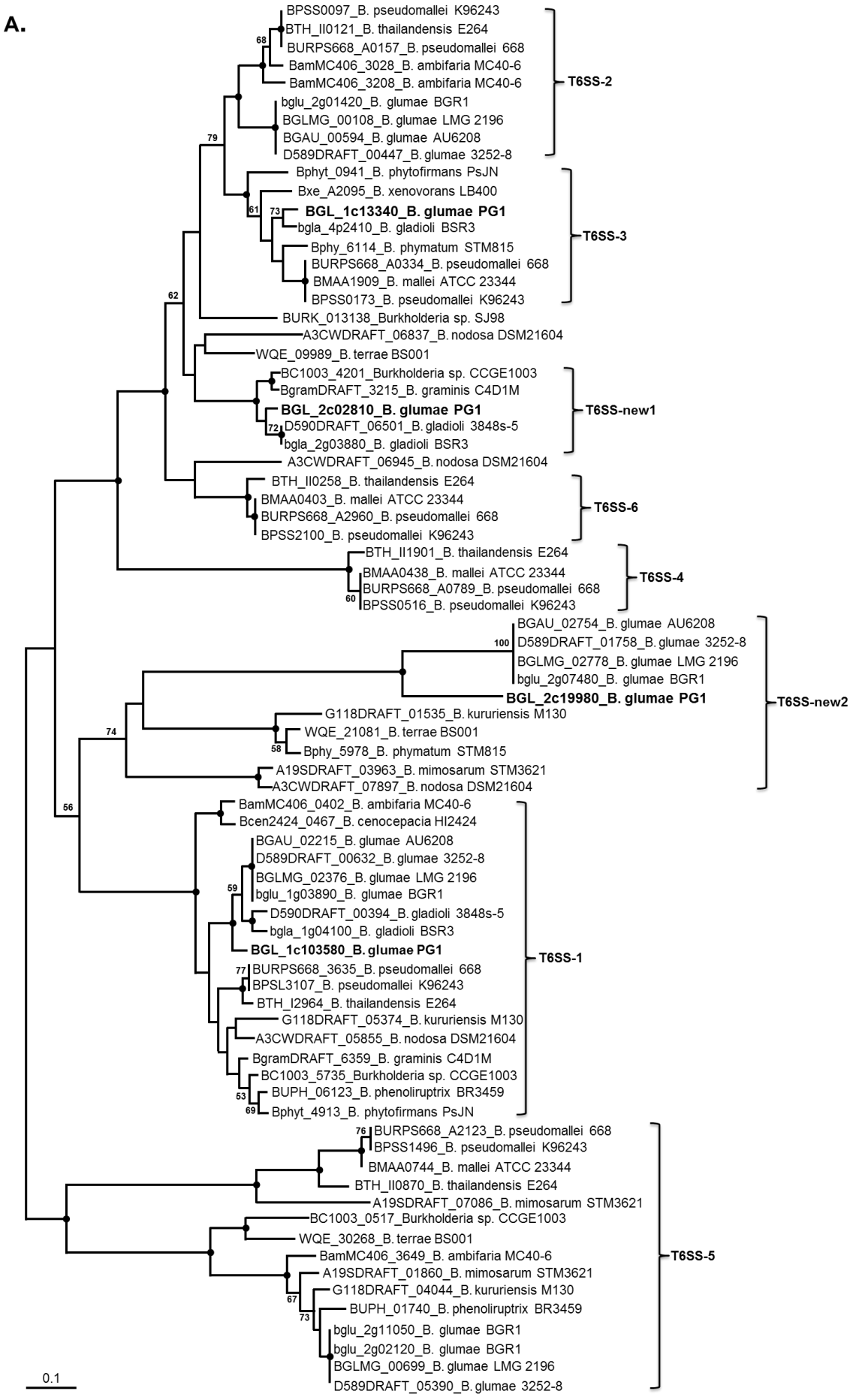
Up to date, only a few studies have been performed using genome wide transcriptome analyses to investigate QS-regulated gene expression patterns for bacteria. Since each study chose a different growth conditions and focused on different organisms, each data set in response to QS processes was different even for the same strain. In some recent studies, the QS regulons represent up to 6.2% of the coding sequences for *P. aeruginosa* (Chugani *et al.*, 2012), up to 8.1 % for *Yersinia pestis* (LaRock *et al.*, 2013), up to 8.0 % for *B. thailandensis*

(Majerczyk *et al.*, 2014a), 0.8 % for *B. mallei*, 3.6 % for *B. pseudomallei* (Majerczyk *et al.*, 2014b) and between 4.9-7.3 % for *Sinorhizobium fredii* NGR234 (Krysciak *et al.*, 2014). Therefore, 11.5 % of differentially regulated genes by three QS systems in this study are reasonable. Remarkably, a recent study showed that up to 19.6 % of the BGR1 genes are regulated in a TofI-QS-dependent manner (Kim *et al.*, 2013). This value is much higher than the present study and other reports since a different cut off was used and the overall read coverage of the BGR1 genome was 8-35-fold lower than in the present study. Also cells were harvested at 8 and 10 h of growth at 37℃ in the BGR1 study (Kim *et al.*, 2013) in contrast to 28 h of growth at 30℃ in this study.

These differentially regulated genes by QS systems in BGPG1 were classified into eleven functional categories based on the KEGG database (Figure 24B-D). Among them, general metabolism category makes up the largest portion of total regulated genes. Type VI Secretion System is a newly described bacterial secretion mechanism which has multiple roles during bacteria life cycle. CRISPR/cas system, which is an adaptive immunity system and also plays roles on bacteria virulence, was first found to be regulated by QS-systems in this study. Thus, the three categories will be further discussed below.

6. T6SS systems are subject to QS-dependent regulation in BGPG1.

Type VI Secretion System (T6SS) was a newly recognized bacterial secretion mechanism by which Gram-negative bacteria translocate proteins directly into prokaryotic or eukaryotic cells in a contact-dependent manner (Mougous *et al.*, 2006; Pukatzki *et al.*, 2006). This secretion system is a new powerful weapon in the bacterial armoury, since it can be used to target either eukaryotic cells or competitor bacteria (Basler *et al.*, 2012; Bernard *et al.*, 2010). It was shown that T6SS can influence virulence, symbiosis, biofilm formation and stress sensing in several bacteria (Aschtgen *et al.*, 2008; Bladergroen, 2003; Mougous *et al.*, 2006; Schwarz *et al.*, 2010; Shyntum *et al.*, 2014; Zheng & Leung, 2007). T6SS is consisted of the core apparatus formed by 13 conserved subunits, and a variable complement of accessory elements, which might facilitate T6SS assembly or confer additional functions to this secretion machines (Cascales & Cambillau, 2012).


A.
BPSS0097_B. pseudomallei K96243
BTH_II0121_B. thailandensis E264
BURPS668_A0157_B. pseudomallei 668
BamMC406_3028_B. ambifaria MC40-6
BamMC406_3208_B. ambifaria MC40-6
bglu_2g01420_B. glumae BGR1
BGLMG_00108_B. glumae LMG 2196
BGAU_00594_B. glumae AU6208
D589DRAFT_00447_B. glumae 3252-8
T6SS-2
Bphyt_0941_B. phytofirmans PsJN
Bxe_A2095_B. xenovorans LB400
BGL_1c13340_B. glumae PG1
bgla_4p2410_B. gladioli BSR3
Bphy_6114_B. phymatum STM815
BURPS668_A0334_B. pseudomallei 668
BMAA1909_B. mallei ATCC 23344
BPSS0173_B. pseudomallei K96243
T6SS-3
BURK_013138_Burkholderia sp. SJ98
A3CWDRAFT_06837_B. nodosa DSM21604
WQE_09989_B. terrae BS001
BC1003_4201_Burkholderia sp. CCGE1003
BgramDRAFT_3215_B. graminis C4D1M
BGL_2c02810_B. glumae PG1
D590DRAFT_06501_B. gladioli 3848s-5
bgla_2g03880_B. gladioli BSR3
T6SS-new1
A3CWDRAFT_06945_B. nodosa DSM21604
BTH_II0258_B. thailandensis E264
BMAA0403_B. mallei ATCC 23344
BURPS668_A2960_B. pseudomallei 668
BPSS2100_B. pseudomallei K96243
T6SS-6
BTH_II1901_B. thailandensis E264
BMAA0438_B. mallei ATCC 23344
BURPS668_A0789_B. pseudomallei 668
BPSS0516_B. pseudomallei K96243
T6SS-4
BGAU_02754_B. glumae AU6208
D589DRAFT_01758_B. glumae 3252-8
BGLMG_02778_B. glumae LMG 2196
bglu_2g07480_B. glumae BGR1
BGL_2c19980_B. glumae PG1
G118DRAFT_01535_B. kururiensis M130
WQE_21081_B. terrae BS001
Bphy_5978_B. phymatum STM815
A19SDRAFT_03963_B. mimosarum STM3621
A3CWDRAFT_07897_B. nodosa DSM21604
T6SS-new2
BamMC406_0402_B. ambifaria MC40-6
Bcen2424_0467_B. cenocepacia HI2424
BGAU_02215_B. glumae AU6208
D589DRAFT_00632_B. glumae 3252-8
BGLMG_02376_B. glumae LMG 2196
bglu_1g03890_B. glumae BGR1
D590DRAFT_00394_B. gladioli 3848s-5
bgla_1g04100_B. gladioli BSR3
BGL_1c103580_B. glumae PG1
BURPS668_3635_B. pseudomallei 668
BPSL3107_B. pseudomallei K96243
BTH_I2964_B. thailandensis E264
G118DRAFT_05374_B. kururiensis M130
A3CWDRAFT_05855_B. nodosa DSM21604
BgramDRAFT_6359_B. graminis C4D1M
BC1003_5735_Burkholderia sp. CCGE1003
BUPH_06123_B. phenoliruptrix BR3459
Bphyt_4913_B. phytofirmans PsJN
T6SS-1
BURPS668_A2123_B. pseudomallei 668
BPSS1496_B. pseudomallei K96243
BMAA0744_B. mallei ATCC 23344
BTH_II0870_B. thailandensis E264
A19SDRAFT_07086_B. mimosarum STM3621
BC1003_0517_Burkholderia sp. CCGE1003
WQE_30268_B. terrae BS001
BamMC406_3649_B. ambifaria MC40-6
A19SDRAFT_01860_B. mimosarum STM3621
G118DRAFT_04044_B. kururiensis M130
BUPH_01740_B. phenoliruptrix BR3459
bglu_2g11050_B. glumae BGR1
bglu_2g02120_B. glumae BGR1
BGLMG_00699_B. glumae LMG 2196
D589DRAFT_05390_B. glumae 3252-8
T6SS-5
0.1

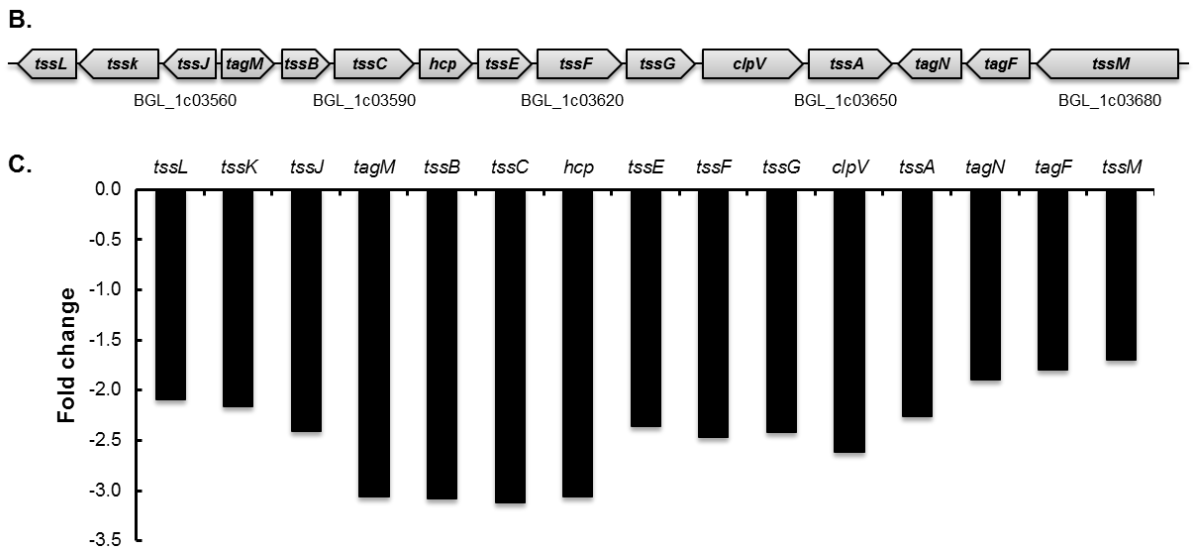


Figure 32. T6SS-1 is QS-regulated in BGPG1. (A) Phylogenetic analysis of T6SSs in the genus *Burkholderia* (This analysis was performed by Dr. Andreas Knapp). The TIGRfam 03358 representing the VC_A0107 family protein (VipA) of T6SS was used to screen 34 *Burkholderia* genomes in the Integrated Microbial Genomes (IMG) database. 77 protein sequences were aligned with clustalW and the genetic tree was established by using the neighbor-joining method and 1000 recalculations by the Bootstrap (BS) test with the default settings of MEGA 6. BS values ≥ 80 are shown as filled circles at the nodes. VipA proteins of *B. glumae* PG1 are highlighted in bold. Known T6SS subtypes named after *B. pseudomallei* T6SS are indicated with numbers. Two new T6SS groups are named as T6SS-new1 and T6SS-new2. **(B)** Genomic organization of T6SS-1 in BGPG1. **(C)** Relative transcript levels of T6SS-1 cluster genes in BGPG2 vs. BGPG1.

B. pseudomallei encode six evolutionarily distinct T6SS gene clusters amed as *Burkholderia* T6SS1-6. Five of them are shared by *B. thailandensis* and *B. mallei* (Schwarz *et al.*, 2010). In this study, a phylogenetic comparison based on VipA, a conserved protein in T6SS, revealed that BGPG1 harbors four of these clusters (Figure 32A). Among them, one cluster present in all other sequenced *B. glumae* strains is homology with *Burkholderia* T6SS-1 (Figure 32A-B). The second cluster located on the chromosome 1 of BGPG1 is similar to *Burkholderia* T6SS-3 (Figure 32A). However, this cluster is absent from all other *B. glumae* strains. The other two T6SS clusters in BGPG1, named as T6SS-new1 and -new2 (Figure 32A), belong to two newly described clades (Fory *et al.*, 2013) and have no similarities with *Burkholderia* T6SSs. T6SS-new1 was supposed to be unique for *B. gladioli* (Fory *et al.*, 2013), but it was also found in *B. graminis* and *Burkholderia sp.* strains. T6SS-new2 is present in all *B. glumae* strains but is absent from the *B. gladioli*. Although *Burkholderia* T6SS-2 and T6SS-5 were predicted to be present in *B. glumae* strains specifically (Fory *et al.*, 2013), they are not present in BGPG1.

Data obtained from a large number of transcriptional profiling studies implicated that QS system is a major regulatory mechanism for T6SS genes expression (Bernard *et al.*, 2010; Khajanchi *et al.*, 2009; Liu *et al.*, 2008). For example, both CepRI and CciRI QS systems in *B. cenocepacia* were reported to regulate gene expression of T6SS clusters, although it is not clear how these two QS systems are connected and how they contribute to T6SS regulation (O'Grady *et al.*, 2009). In this work, RNA-seq data indicated that T6SS-affiliated and QS-regulated genes spreading up in several clusters were identified on both chromosomes. In *bgaI1* mutant BGPG2, all genes encoding for T6SS-1 (BGL_1c03540 to BGL_1c03650) were 2.1-3.1-fold down-regulated (Figure 25A cluster 10; Figure 32C; Table 18). Given that several recent studies showed a critical role of T6SS-1 in the virulence of *B. mallei* and *B. pseudomallei* (Burtnick *et al.*, 2010, 2011; Pilatz *et al.*, 2006; Schell *et al.*, 2007), it is reasonable to hypothesize that T6SS-1 plays a role in virulence of BGPG1. Additionally, T6SS-1 was shown to be necessary for inter-bacteria competition for *B. thailandensis* (Miyata *et al.*, 2013; Schwarz *et al.*, 2010, 2014), highlighting the importance of this system in bacteria lifestyle.

7. CRISPR-Cas system is subject to QS-dependent regulation in BGPG1.

CRISPR-Cas (clustered regularly interspaced short palindromic repeats–CRISPR-associated proteins) system is an adaptive immunity system that are observed in many bacteria and archaea (Bhaya *et al.*, 2011) (Figure 33A). This system protects prokaryotes from invasions of a wide range of mobile genetic elements, such as viruses and plasmids (Richter *et al.*, 2014). Based on currently obtained sequencing data of *cas* genes and phylogenetic studies, CRISPR–Cas systems are classified into three major types, type I, type II and type III (Makarova *et al.*, 2011). Type I system is characterized by its own signature protein Cas3, type II by Cas9 and type III by Cas10 (Makarova *et al.*, 2011). Each main type is further classified into several subtypes by their unique subset of proteins. Cas1 and cas2 are regarded as universal markers which are present in all CRISPR-Cas systems.

BGPG1 encodes a single CRISPR-Cas system in chromosome 1 which is classified into F subtype of type I (formerly Ypest) by signature protein of Csy1,Cys2, Cys3 and Cys4 (Figure

33B) (Voget *et al.*, 2015). It includes three CRISPR arrays, and the *cas* operon encoding Cas1, Cas3 and subtype signature proteins Cys1-4. Two CRISPR arrays surround the *cas* genes, with a preceding locus consisting of 14 repeats and a latter locus consisting of 25 repeats. Another array is located 300 kb downstream of the second array. Each repeat is interrupted by spacers with a length of 32-33 nucleotides. The functional screening with the cas-associated TIGRfams (in total 101) in the genomes of all other sequenced *B. glumae* strains (Performed by Dr. Andreas Knapp, data not pluclished) indicated that the CRISPR-Cas system has been only founded in strain 3252-8, which was also classified as type I-F, not in 336gr-1, AU6208, LMG2196 and the extensively-studied strain BGR1. In this work, transcriptome analysis data indicated that all CRISPR-Cas system related genes spanning from BGL_1c18810 to BGL_1c18860 were differentially transcribed in the three AHL mutants compared to the parent strain BGPG1 (Figure 25A cluster 10; Figure 33C; Table 18). Specifically, there was a 2-9.5-fold decreased transcription in BGPG2, a 1.2-3.9-fold down-regulation in BGPG3 and a 1.1-5.5-fold decreased transcription in BGPG4. Data from qRT-PCR analyses supported these findings (Figure 26).

The roles of CRISPR-Cas system in host immunity is extensively studied, and increasing evidence suggests that this mechanism may have effects on many bacterial physiology processes, like virulence and heterochromatin formation (Gunderson & Cianciotto, 2013; Richter & Fineran, 2013; Richter *et al.*, 2014; Sampson *et al.*, 2013; Westra *et al.*, 2014). CRISPR-cas system was demonstrated to be necessary for pathogen *C. jejuni* (Louwen *et al.*, 2013)and *F. novicida* (Sampson *et al.*, 2013) to successfully infect host cells. This is because that CRISPR-Cas system in both pathogens plays a role to inhibit the expression of virulence factors, resulting in escaping from recognition by host immune system and replicating in host (Louwen *et al.*, 2013; Sampson *et al.*, 2013). Similar results were obtained in another strain *P. aeruginosa*, in which the CRISPR-Cas type I-F system is involved in viral defence and also could be involved in regulation of virulence gene expression in *P. aeruginosa* lysogen for bacteriophage DMS3 (Palmer & Whiteley, 2011; Zegans *et al.*, 2009). Richter and colleagues addressed that the subtype I-F CRISPR-Cas system in *P. atrosepticum* can induce the chromosomal targeting, resulting in substantial rearrangements within the genome (Richter &

Fineran, 2013). The rearrangements in the end will influence the pathogenicity island retention.

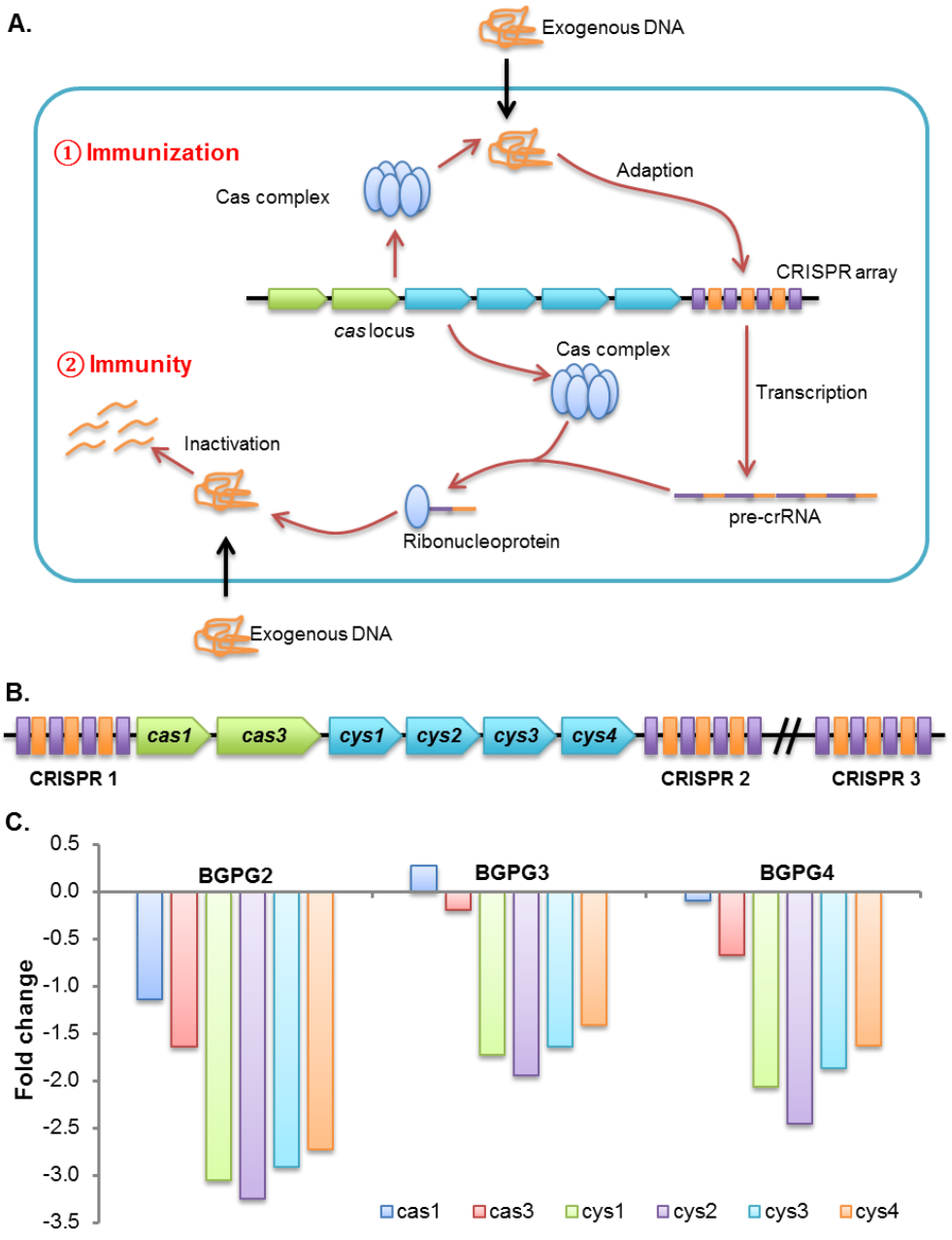


Figure 33. CRISPR-Cas system is QS-regulated in BGPG1. (A) Working model of CRISPR-Cas system. Immunization phase: the exogenous DNA, such as virus DNA and plasmid DNA, is acquired by Cas complex and their signature fragments are stored into the CRISPR arrays. Immunity phase: Pre-CRISPR RNA (Pre-crRNA) transcribed from the CRISPR array is cleaved by Cas complex to form a mature crRNA, which combines with a cas protein to form a ribonucleoprotein complex. The crRNA in the ribonucleoprotein contains a spacer that has a strong match to the exogenous DNA and initiates a cleavage, resulting in the inactivation of the exogenous DNA **(B)** Schematic genomic organization of Subtype I-F CRISPR-Cas system in BGPG1. The *cas* operon encodes Cas1, Cas3, and the subtype-specific Csy1, Csy2, Csy3 and Cas4 proteins. The *cas* operon is flanked by CRISPR1 and CRISPR2 and CRISPR3 (downstream). CRISPR3 is located about 300 kb downstream of CRISPR2.

(C) Relative transcript levels of CRISPR-Cas cluster genes in BGPG2-4 vs. BGPG1.

CRISPR-Cas system plays multiple roles during bacteria lifecycle. On the one hand, bacteria make use of this system as a part of immunity system to resist invasions from motile genetic elements, such as virus and plasmids. On the other hand, bacteria require this system to cover their nature properties during infection and thus obtain an efficient invasion in host. The molecular mechanisms behind these processes are poorly defined. Notably, this study firstly showed that QS systems in BGPG1 play a role on regulating CRISPR-Cas genes expression. Since other *B. glumae* isolates don't encode the CRISPR-Cas system, it suggests that strains 3252-8 and BGPG1 have more complicated lifestyles. Therefore, it would be interesting to investigate the interaction mechanisims between QS-systems and CRISPR-cas system which may help to obtain a better understanding of complicated lifestyle of BGPG1 and the mechanisms of how CRISPR-Cas plays its double roles.

8. Genes linked to metabolic activities are QS-regulated in BGPG1.

QS-process allows bacteria to monitor their population and coordinate their group behaviour through regulation of gene expression, many of which are relative to the metabolic activities in many species of *Proteobacteria*. QS-system modulates metabolic activities to ensure the metabolic equilibrium in individual cells at different population situation, which is an important form of cooperative activity. Recently, it was reported that QS has effects on the regulation of carbon and energy source metabolism in *Y. pestis, A. hydrophila*, *S. plymuthica* and *S. marcescens* (Van Houdt *et al.*, 2006, 2007; LaRock *et al.*, 2013), the antibiotic synthesis in *E. carotovora*, fluorescent *Pseudomonas* Spp, and *Lactococcus lactis* (Kleerebezem, 2004; Mavrodi *et al.*, 2006; McGowan *et al.*, 2005), the metabolism of pollutants in *P. aeruginosa* and *Acinetobacter* sp (Kang & Park, 2010; Yong & Zhong, 2010) and amino acids metabolism in *V. campbellii* and *C. jejuni* (Quiñones *et al.*, 2009; Wang *et al.*, 2013).

Members of the genus *Burkholderia* was generally regarded as prolific producers of secondary metabolites. 25 secondary metabolite gene clusters were identified in the genome of *B. glumae* PG1 using genome-guided approaches by Knapp and co-operators (Knapp *et al.*, 2015). Interestingly, RNA-seq data of BGPG1 in this study revealed that nearly 40% of all

QS-regulated genes were linked to the metabolic activities (Figure 24). Notably, several clusters involved in the polyketide biosynthesis, non-ribosomal peptide biosynthesis and rhamnolipid biosynthesis (Figure 25B cluster 6; Table 18, discussed in VI 2.4) were QS regulated, which are consistent with results obtained from *B. thailandensis* (Majerczyk *et al.*, 2014a) and *P. aeruginosa* (Déziel *et al.*, 2005; Williamson *et al.*, 2008).

Moreover, genes involved in the phosphate metabolism (Figure 25B, cluster 5; Table 18) as well as a large cluster involved in inositol phosphate biosynthesis (Figure 25A cluster 7; Table 18) were also QS-regulated in BGPG1. Specifically, the *bgaI1*-deletion resulted in a 2.0-3.4-fold up-regulation of *iolBCDE* and *idhA*, which are relative to inositol phosphate metabolism pathway. The deletion of each *bgaI* gene resulted in a 2.1-14.3-fold down-regulation of genes *phnWSTUVA* encoded in the cluster BGL_2c04810- BGL_2c04860, which are responsible for phosphate metabolism and conserved in several *Burkholderia* species, including *B. pseudomallei*, *B. mallei* and *B. cepacia*.

In addition, several clusters involved in cofactor biosynthesis were QS-regulated (Figure 25A, cluster 11; Figure 25B, cluster 9; Table 18). The third redox cofactor, Pyrroloquinoline quinone (PQQ), is a common one of them. PQQ is involved in the primary oxidation of growth substrates such as alcohols, amines, and aldose sugars and is required for stimulating the growth in bacteria (Ameyama *et al.*, 1988), In BGPG1, one operon *pqqBCDE* together with two *pqqC* solo genes is supposed to be responsible for PQQ biosynthesis. RNA-seq data indicated that in all *bgaI* mutants, the operon *pqqBCDE* is modestly down-regulated. However, in the *bgaI3* deletion mutant, the *pqqBC* were 2.1-fold down-regulated. Given that *pqqBC* genes are located upstream of *pqqDE*, it is reasonable to conclude that PQQ was at least regulated by BgaI-3-QS system in BGPG1. Since there is no study about the connection of PQQ and QS in *B. glumae*, this finding could be an interesting starting about research in this field.

Considering the relatively high number of QS-regulated genes linked to metabolite biosynthesis and the general metabolism, one can speculate that QS is of high importance for organisms to survive and grow in soil or sustain saprophytic growth on plant surface.

9. QS-regulated orthologous in other *Burkholderia* species.

QS-processes, serving as one of the fundamental requirements for bacterial survival, confer bacteria the ability to quickly adapt to the changes of lifestyles at a population level. Since *B. glumae* is a soil-associated plant pathogen, the following question was asked, which of the QS-regulated genes are associated with life in soil and which ones are involved in plant infection. For this purpose, it is valuable to make a cross-species analysis of QS-regulated genes between BGPG1 and other strains with different lifestyles. Recent studies performed by Majerczyk and colleagues (Majerczyk *et al.*, 2014a, b) have described the QS regulons in the tropical soil bacterium *B. thailandensis,* host restricted pathogenic species *B. mallei* and human pathogenic species *B. pseudomallei.* Among them*, B. mallei* encode two functional LuxIR-type pairs which are conserved in other two strains. *B. thailandensis* and *B. pseudomallei* contain one more QS system which does not exist in *B. mallei* (Majerczyk *et al.*, 2014b).

A direct comparison of OS-regulated genes between BGPG1 and *B. thailandensis* revealed that a common set of 61 were co-regulated in both microbes (Majerczyk *et al.*, 2014a). Among them, 41 genes were regulated by *bgaI1* in BGPG1, 17 genes by *bgaI2* and 26 by *bgaI3.* 8 QS-regulated genes were found to be shared by *B. pseudomallei* and BGPG1 through comparison with data published by Majerczyk *et al* (Majerczyk *et al.*, 2014b), however, no QS-regulated genes in BGPG1 are shared with *B. mallei* (Majerczyk *et al.*, 2014b). Furthermore, no common core set of QS-regulated genes is shared by all four *Burkholderia* strains. And only a small number of QS-regulated genes are shared by *B. thailandensis*, *B. pseudomallei* and BGPG1. Therefore, it is reasonable to conclude that QS system modulate different bacteria lifestyles through control expression of different genes. And in the case of BGPG1, the 61 QS-regulated genes shared with *B. thailandensis* are promising candidates associated with bacteria life in soil, since both strains are soil associated. And the 8 QS-regulated genes shared with *B. pseudomallei* could be possible candidates for human pathogenicity, although only a single case was reported of *B. glumae* isolated from a clinical human sample (Devescovi *et al.*, 2007). Given that there is no any similarity of lifestyles between *B. mallei* and other three strains, it is make sense of the absence of one common set

of QS-regulated genes in these strains, which also suggesting a wider phylogenetic distance between *B. mallei* and BGPG1.

Table 17. Shared function of QS-regulated homologues between BGPG1 and the *Bptm* group.

Shared functional homologues genes	BG	BT	BP	BM
Flagella	+ (21)	+ (6)	-	-
AI synthase	+ (3)	+ (3)	+ (1)	-
LuxR protein	+ (5)	+ (3)	-	+ (1)
Polyketide biosynthesis (PKS)	+ (5)	+ (7)	+ (6)	+ (2)
Non-ribosomal peptide synthase (NRPS)	+ (5)	+ (1)	+ (2)	+ (2)
Rhamnosyltransferase I	+ (3)	+ (2)	-	-
Type I secretion	+ (2)	+ (1)	-	-
Type II secretion	-	+ (1)	-	-
Type III secretion	+ (4)	+ (1)	+ (3)	-
Type IV secretion	-	+ (2)	-	-
Type VI secretion	+ (13)	-	-	-
Flp Pilus	+ (9)	-	-	-
Lipase A	+ (1)	-	-	-
Histidine Utilization System	+ (3)	+ (3)	+ (1)	-
Phosphate metabolism	+ (6)	-	-	-
Major facilitator family transporter	+ (10)	+ (5)	+ (4)	-
ABC transporter	+ (17)	+ (14)	+ (7)	+ (2)
Polysaccharide biosynthesis	+ (6)	+ (6)	+ (1)	-
Lipoprotein	+ (11)	+ (8)	+ (1)	-
Ribosomal protein	+ (7)	+ (1)	+ (26)	-

*BT, *B. thailandensis*; BP, *B. pseudomallei*; BM, *B. mallei*. Transcriptome data for the *Bptm* group (BT, BP, BM) were obtained from references (Majerczyk *et al.*, 2014a, b) and identified homologous genes were given by numbers in brackets.

To further understand this finding, the functional comparison was performed based on the functions of QS-regulated genes among four *Burkholderia* strains. In total, 20 functional categories were classified and 14 were found to be shared at least by two strains (Table 17).

Among them, 14 categories of BGPG1 are shared with *B. thailandensis*, 10 with *B. pseudomallei* and only 3 with *B. mallei*. These findings further confirmed that QS-regulated genes have more similarities among strains with close lifestyles. Interestingly, 3 functional categories were shared by all four strains, namely non-ribosomal peptide synthase (NRPS), polyketide synthase (PKS) and ABC transporter (Table 17). Of these, NRPS and PKS are the hallmarks of secondary metabolites (Shen *et al.*, 2001). Since the genus *Burkholderia* is known to be prolific producers of secondary metabolites (Knapp *et al.*, 2015), it is reasonable that NRPS and PKS associated genes are QS-regulated in four *Burkholderia* strains. Notably, rhamnolipid, the important biosurfactant and secondary metabolite, is QS-independent in three *Bptm* strains, which could be because they are not plant-associated bacteria.

10. Conclusion and future direction

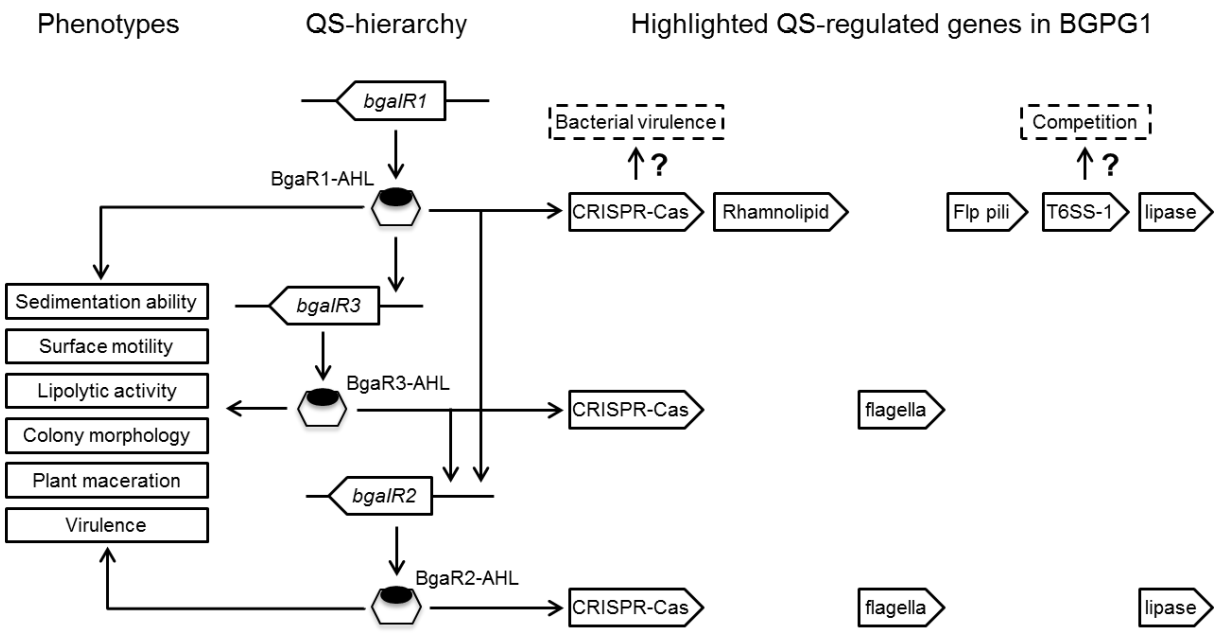


Figure 34. QS-hierarchy in B. glumae PG1 modulates bacterial lifestyle.

The main objective of this study was to investigate the functions of quorum sensing systems in *B. glumae* strain PG1. In this work, three distinct and functional *luxI* homologues *bgaI1-3*, were characterized in BGPG1 and their roles in bacterial lifestyle were investigated. A model describing roles of *bgaI1-3* QS systems are present in Figure 34. The *bgaI1-3* QS systems formed a hierarchy with *bgaI1* system at the top followed by *bgaI3* system in BGPG1. This

hierarchy controls surface motility, sedimentation ability, lipolytic activity, colony morphology, plant maceration and rice pathogenicity of BGPG1. Genome-wide transcriptome analysis revealed that a total of 745 genes in BGPG1 were QS-regulated. The majority of them were linked to metabolic activities and the most pronounced regulation was observed for the genes involved in rhamnolipid, lipase and Flp pili biosynthesis, flagella associated genes, the type VI secretion system and genes linked to a CRIPSR-*cas* gene cluster. In addition, the cross-species analysis of QS-regulated genes between BGPG1 and all members of *Bptm* group suggested that BGPG1 has more similarities with *B. thailandensis* than other two *Bptm* strains, *B. pseudomallei* and *B. mallei* in lifestyle. These findings could contribute to understand the lifestyle of *B. glumae* PG1.

Multiple QS-systems in *B. glumae* PG1 is much more complicated than single QS-system in other *B. glumae* isolates. They could be a useful and challengeable model to study pathogenicity mechanisms and lifestyle of *B. glumae*. CRISPR-Cas is a prokaryotic immune system, which also plays important roles on many bacterial physiology processes like virulence. It was newly identified to be regulated by QS-systems in this study. T6SS was also a newly identified bacterial secretion mechanism which was involved in virulence and biofilm formation. Even though it has been demonstrated that these two systems are very important for bacteria lifecycle, their molecular mechanisms are poorly defined. Therefore, the future work will focus on the investigation of molecular mechanisms behind the interaction between *bgaI*-QS systems and both CRISPR-Cas and T6SS systems, especially their roles on bacteria virulence and competition with other bacteria.

V. References

Aguirre-Ramírez, M., Medina, G., González-Valdez, A., Grosso-Becerra, V. & Soberón-Chávez, G. (2012). The Pseudomonas aeruginosa rmlBDAC operon, encoding dTDP-L-rhamnose biosynthetic enzymes, is regulated by the quorum-sensing transcriptional regulator RhlR and the alternative sigma factor σS. *Microbiology* **158**, 908–16.

Ahmad, I., Ahmad, F. & Pichtel, J. (2011). *Microbes and Microbial Technology*.

Aldridge, P. & Hughes, K. (2002). Regulation of flagellar assembly. *Curr Opin Microbiol* 160–165.

Ameyama, M., Matsushita, K., Shinagawa, E., Hayashi, M. & Adachi, O. (1988). Pyrroloquinoline quinone: excretion by methylotrophs and growth stimulation for microorganisms. *Biofactors* **1**, 51–53. ENGLAND.

Ansaldi, M., Marolt, D., Stebe, T., Mandic-Mulec, I. & Dubnau, D. (2002). Specific activation of the Bacillus quorum-sensing systems by isoprenylated pheromone variants. *Mol Microbiol* **44**, 1561–73.

Aparna, G., Chatterjee, A., Sonti, R. V & Sankaranarayanan, R. (2009). A cell wall-degrading esterase of Xanthomonas oryzae requires a unique substrate recognition module for pathogenesis on rice. *Plant Cell* **21**, 1860–73.

Arpigny, J. L. & Jaeger, K. E. (1999). Bacterial lipolytic enzymes: classification and properties. *Biochem J* **343 Pt 1**, 177–183.

Aschtgen, M.-S., Bernard, C. S., De Bentzmann, S., Lloubès, R. & Cascales, E. (2008). SciN is an outer membrane lipoprotein required for type VI secretion in enteroaggregative Escherichia coli. *J Bacteriol* **190**, 7523–31.

Atkinson, S., Chang, C., Sockett, R. E., Ca, M., Williams, P. & Acteriol, J. B. (2006). Quorum Sensing in Yersinia enterocolitica Controls Swimming and Swarming Motility **188**, 1451–1461.

Balkenhohl, F., Ditrich, K., Hauer, B. & Ladner, W. (1997). Optically Active Amines via Lipase-Catalyszed Methoxyacetylation. *J Prakt Chem* **339**, 381–384.

Basler, M., Pilhofer, M., Henderson, G. P., Jensen, G. J. & Mekalanos, J. J. (2012). Type VI secretion requires a dynamic contractile phage tail-like structure. *Nature* **483**, 182–6. Nature Publishing Group.

Bassler, B. L., Wright, M. & Silverman, M. R. (1994). Multiple signalling systems controlling expression of luminescence in Vibrio harveyi: sequence and function of genes encoding a second sensory pathway. *Mol Microbiol* **13**, 273–86.

Beisson, F., Tiss, A., Rivière, C. & Verger, R. (2000). Methods for lipase detection and assay: a critical review. *Eur J Lipid Sci Technol* **102**, 133–153.

Bernard, C. S., Brunet, Y. R., Gueguen, E. & Cascales, E. (2010). Nooks and crannies in type VI secretion regulation. *J Bacteriol* **192**, 3850–60.

Bertin, P., Terao, E., Lee, E. U. N. H. E. E., Lejeune, P., Colson, C., Danchin, A. & Collatz, E. (1994). The H-NS Protein Is Involved in the Biogenesis of Flagella in Escherichia coli **176**, 5537–5540.

Bhaya, D., Davison, M. & Barrangou, R. (2011). CRISPR-Cas systems in bacteria and archaea: versatile small RNAs for adaptive defense and regulation. *Annu Rev Genet* **45**, 273–97.

Bijtenhoorn, P., Mayerhofer, H., Müller-Dieckmann, J., Utpatel, C., Schipper, C., Hornung, C., Szesny, M., Grond, S., Thürmer, A. & other authors. **(2011, January).** A novel metagenomic short-chain dehydrogenase/reductase attenuates Pseudomonas aeruginosa biofilm formation and virulence on Caenorhabditis elegans. *PLoS One*.

Bladergroen, M. (2003). Infection-blocking genes of a symbiotic Rhizobium leguminosarum strain that are involved in temperature-dependent protein secretion. *Mol plant-microbe …* **16**, 53–64.

Blosser, R. S. & Gray, K. M. (2000, March). Extraction of violacein from Chromobacterium violaceum provides a new quantitative bioassay for N-acyl homoserine lactone autoinducers. *J Microbiol Methods*.

Bolger, A., Lohse, M. & Usadel, B. (2014). Trimmomatic: a flexible trimmer for Illumina sequence data. *Bioinformatics* 1–7.

Burtnick, M. N., DeShazer, D., Nair, V., Gherardini, F. C. & Brett, P. J. (2010). Burkholderia mallei cluster 1 type VI secretion mutants exhibit growth and actin polymerization defects in RAW 264.7 murine macrophages. *Infect Immun* **78**, 88–99.

Burtnick, M. N., Brett, P. J., Harding, S. V, Ngugi, S. a, Ribot, W. J., Chantratita, N., Scorpio, A., Milne, T. S., Dean, R. E. & other authors. **(2011).** The cluster 1 type VI secretion system is a major virulence determinant in Burkholderia pseudomallei. *Infect Immun* **79**, 1512–25.

Caiazza, N., Shanks, R. & O'Toole, G. (2005). Rhamnolipids modulate swarming motility patterns of Pseudomonas aeruginosa. *J Bacteriol* **187**.

Cascales, E. & Cambillau, C. (2012). Structural biology of type VI secretion systems. *Philos Trans R Soc Lond B Biol Sci* **367**, 1102–11.

Chatterjee, A., Cui, Y., Chakrabarty, P. & Chatterjee, A. K. (2010). Regulation of Motility in Erwinia carotovora subsp . carotovora : Quorum-Sensing Signal Controls FlhDC , the Global Regulator of Flagellar and Exoprotein Genes , by Modulating the Production of RsmA , an RNA-Binding Protein **23**, 1316–1323.

Chen, H., An, R., Tang, J.-H., Cui, X.-H., Hao, F.-S., Chen, J. & Wang, X.-C. (2007). Over-expression of a vacuolar Na+/H+ antiporter gene improves salt tolerance in an upland rice. *Mol Breed* **19**, 215–225.

Chen, I. & Dubnau, D. (2004). DNA uptake during bacterial transformation. *Nat Rev Microbiol* **2**, 241–9.

Chen, R., Barphagha, I. K., Karki, H. S. & Ham, J. H. (2012, January). Dissection of quorum-sensing genes in Burkholderia glumae reveals non-canonical regulation and the new regulatory gene tofM for toxoflavin production. *PLoS One*.

Chester, N. & Marshak, D. R. (1993, March). Dimethyl sulfoxide-mediated primer Tm reduction: a method for analyzing the role of renaturation temperature in the polymerase chain reaction. *Anal Biochem*.

Chevance, F. F. V & Hughes, K. T. (2008). Coordinating assembly of a bacterial macromolecular machine. *Nat Rev Microbiol* **6**, 455–65.

Chien, C. C.; Chang, Y. C. (1987). The susceptibility of rice plants at different growth stages and of 21 commercial rice varieties to Pseudomonas glumae. *J Agric Res China* **36**, 302–310.

Christensen, A. B., Riedel, K., Eberl, L., Flodgaard, L. R., Molin, S., Gram, L. & Givskov, M. (2003). Quorum-sensing-directed protein expression in Serratia proteamaculans B5a. *Microbiology* **149**, 471–483.

Chugani, S., Kim, B. S., Phattarasukol, S., Brittnacher, M. J., Choi, S. H., Harwood, C. S. & Greenberg, E. P. (2012). Strain-dependent diversity in the Pseudomonas aeruginosa quorum-sensing regulon. *Proc Natl Acad Sci U S A* **109**, E2823–31.

Chun, H., Choi, O., Goo, E., Kim, N., Kim, H., Kang, Y., Kim, J., Moon, J. S. & Hwang, I. (2009). The quorum sensing-dependent gene katG of Burkholderia glumae is important for protection from visible light. *J Bacteriol* **191**, 4152–7.

Clough, S. J., Lee, K. E., Schell, M. A. & Denny, T. P. (1997). A two-component system in Ralstonia (Pseudomonas) solanacearum modulates production of PhcA-regulated virulence factors in response to 3-hydroxypalmitic acid methyl ester. *J Bacteriol* **179**, 3639–3648.

Coenye, T. & Vandamme, P. (2003). Diversity and significance of Burkholderia species occupying diverse ecological niches. *Environ Microbiol* **5**, 719–729.

Cooper, V. S., Vohr, S. H., Wrocklage, S. C. & Hatcher, P. J. (2010). Why genes evolve faster on secondary chromosomes in bacteria. *PLoS Comput Biol* **6**, e1000732.

Costa, S. G. V. a O., Déziel, E. & Lépine, F. (2011). Characterization of rhamnolipid production by Burkholderia glumae. *Lett Appl Microbiol* **53**, 620–7.

Cottyn, B., Outryve, M. Van & Cerez, M. (1996). Bacterial diseases of rice. II. Characterization of pathogenic bacteria associated with sheath rot complex and grain discoloration of rice in the Philippines. *Plant Dis*.

Coutinho, B. G., Mitter, B., Talbi, C., Sessitsch, A., Bedmar, E. J., Halliday, N., James, E. K., Cámara, M. & Venturi, V. (2013). Commonalities and Differences in Regulon studies and in planta role of the BraI/R quorum-sensing system in the plant-beneficial Burkholderia cluster. *Appl Environ Microbiol* **79**, 4421–32.

Craig, L., Pique, M. E. & Tainer, J. a. **(2004).** Type IV pilus structure and bacterial pathogenicity. *Nat Rev Microbiol* **2**, 363–78.

Degrassi, G., Devescovi, G., Kim, J., Hwang, I. & Venturi, V. (2008). Identification, characterization and regulation of two secreted polygalacturonases of the emerging rice pathogen Burkholderia glumae. *FEMS Microbiol Ecol* **65**, 251–62.

Dehio, C. & Meyer, M. (1997). Maintenance of broad-host-range incompatibility group P and group Q plasmids and transposition of Tn5 in Bartonella henselae following conjugal plasmid transfer from Escherichia coli. *J Bacteriol* **179**, 538–40.

Deng, Y., Wu, J., Eberl, L. & Zhang, L. H. (2010). Structural and functional characterization of diffusible signal factor family quorum-sensing signals produced by members of the burkholderia cepacia complex. *Appl Environ Microbiol* **76**, 4675–4683.

Deng, Y., Wu, J., Tao, F. & Zhang, L. H. (2011). Listening to a new language: DSF-based quorum sensing in gram-negative bacteria. *Chem Rev* **111**, 160–179.

Devescovi, G., Bigirimana, J., Degrassi, G., Cabrio, L., LiPuma, J. J., Kim, J., Hwang, I. & Venturi, V. (2007). Involvement of a quorum-sensing-regulated lipase secreted by a clinical isolate of Burkholderia glumae in severe disease symptoms in rice. *Appl Environ Microbiol* **73**, 4950–8.

Deziel, E. (2003). rhlA is required for the production of a novel biosurfactant promoting swarming motility in Pseudomonas aeruginosa: 3-(3-hydroxyalkanoyloxy)alkanoic acids (HAAs), the precursors of rhamnolipids. *Microbiology* **149**, 2005–2013.

Déziel, E., Gopalan, S., Tampakaki, A. P., Lépine, F., Padfield, K. E., Saucier, M., Xiao, G. & Rahme, L. G. (2005). The contribution of MvfR to Pseudomonas aeruginosa pathogenesis and quorum sensing circuitry regulation: multiple quorum sensing-regulated genes are modulated without affecting lasRI, rhlRI or the production of N-acyl-L-homoserine lactones. *Mol Microbiol* **55**, 998–1014.

Diggle, S. P., Winzer, K., Chhabra, S. R., Worrall, K. E., Cámara, M. & Williams, P. (2003). The Pseudomonas aeruginosa quinolone signal molecule overcomes the cell density-dependency of the quorum sensing hierarchy, regulates rhl-dependent genes at the onset of stationary phase and can be produced in the absence of LasR. *Mol Microbiol* **50**, 29–43.

Diggle, S. P., Matthijs, S., Wright, V. J., Fletcher, M. P., Chhabra, S. R., Lamont, I. L., Kong, X., Hider, R. C., Cornelis, P. & other authors. **(2007).** The Pseudomonas aeruginosa 4-Quinolone Signal Molecules HHQ and PQS Play Multifunctional Roles in Quorum Sensing and Iron Entrapment. *Chem Biol* **14**, 87–96.

Dobson, A., Cotter, P. D., Ross, R. P. & Hill, C. (2012). Bacteriocin production: a probiotic trait? *Appl Environ Microbiol* **78**, 1–6.

Dubeau, D., Déziel, E., Woods, D. E. & Lépine, F. (2009). Burkholderia thailandensis harbors two identical rhl gene clusters responsible for the biosynthesis of rhamnolipids. *BMC Microbiol* **9**, 263.

Duerkop, B. A., Ulrich, R. L. & Greenberg, E. P. (2007). Octanoyl-Homoserine Lactone Is the Cognate Signal for Burkholderia mallei BmaR1-BmaI1 Quorum Sensing. *J Bacteriol* **189**, 5034–5040.

Duerkop, B. A., Herman, J. P., Ulrich, R. L., Churchill, M. E. A. & Greenberg, E. P. (2008). The Burkholderia mallei BmaR3-BmaI3 Quorum-Sensing System Produces and Responds to N-3-Hydroxy-Octanoyl Homoserine Lactone. *J Bacteriol* **190**, 5137–5141.

Eberhard, A., Burlingame, A. L., Eberhard, C., Kenyon, G. L., Nealson, K. H. & Oppenheimer, N. J. (1981). Structural identification of autoinducer of Photobacterium fischeri luciferase. *Biochemistry* **20**, 2444–2449.

Eberhard, A., Longin, T., Widrig, C. A. & Stranick, S. J. (1991). Synthesis of the lux gene autoindueer in Vibrio fischeri is positively autoregulated 294–297.

Eberl, L. (2006, April). Quorum sensing in the genus Burkholderia. *Int J Med Microbiol*.

Engebrecht, J. & Silverman, M. (1984). Identification of genes and gene products necessary for bacterial bioluminescence. *Proc Natl Acad Sci U S A* **81**, 4154–8.

Estrada-de los Santos, P., Vinuesa, P., Martínez-Aguilar, L., Hirsch, A. M. & Caballero-Mellado, J. (2013). Phylogenetic analysis of burkholderia species by multilocus sequence analysis. *Curr Microbiol* **67**, 51–60.

Fast, W. & Tipton, P. a. **(2012).** The enzymes of bacterial census and censorship. *Trends Biochem Sci* **37**, 7–14. Elsevier Ltd.

Feng, J., Liu, G., Selvaraj, G., Hughes, G. R. & Wei, Y. (2005). A secreted lipase encoded by LIP1 is necessary for efficient use of saturated triglyceride lipids in Fusarium graminearum. *Microbiology* **151**, 3911–21.

Fory, P. A., Triplett, L., Ballen, C., Abello, J. F., Duitama, J., Aricapa, M. G., Prado, G. A., Correa, F., Hamilton, J. & other authors. **(2013).** Comparative Analysis of Two Emerging Rice Seed Bacterial Pathogens. *Phytopathology* **104**, 436–444. Scientific Societies.

Frenken, L. G., Egmond, M. R., Batenburg, A. M., Bos, J. W., Visser, C. & Verrips, C. T. (1992). Cloning of the Pseudomonas glumae Lipase Gene and Determination of the Active Site Residues. *Appl ….*

Fuqua, C. & Greenberg, E. P. (2002). Listening in on bacteria: acyl-homoserine lactone signalling. *Nat Rev Mol Cell Biol* **3**, 685–95.

Fuqua, W. C. & Winans, S. C. (1994, May). A LuxR-LuxI type regulatory system activates Agrobacterium Ti plasmid conjugal transfer in the presence of a plant tumor metabolite. *J Bacteriol*.

Galeva, A., Moroz, N., Yoon, Y.-H., Hughes, K. T., Samatey, F. a & Kostyukova, A. S. (2014). Bacterial flagellin-specific chaperone FliS interacts with anti-sigma factor FlgM. *J Bacteriol* **196**, 1215–21.

Gamage, A. M., Shui, G., Wenk, M. R. & Chua, K. L. (2011, April). N-Octanoylhomoserine lactone signalling mediated by the BpsI-BpsR quorum sensing system plays a major role in biofilm formation of Burkholderia pseudomallei. *Microbiology*.

Gao, R., Krysciak, D., Utpatel, C., Knapp, A., Schmeisser, C., Daniel, R., Voget, S., Jaeger, K.-E. & Streit, W. R. (2015). Genome-wide RNA sequencing analysis of quorum sensing-controlled regulons in the plant-associated Burkholderia glumae strain PG1. *Appl Environ Microbiol* Submitted.

Glessner, A. & Smith, R. (1999). Roles of Pseudomonas aeruginosa las andrhl Quorum-Sensing Systems in Control of Twitching Motility. *J ….*

Goo, E., Majerczyk, C. D., An, J. H., Chandler, J. R., Seo, Y.-S., Ham, H., Lim, J. Y., Kim, H., Lee, B. & other authors. **(2012).** Bacterial quorum sensing, cooperativity, and anticipation of stationary-phase stress. *Proc Natl Acad Sci U S A* **109**, 19775–80.

Goto, K., Ohata, K. (1956). New bacterial diseases of rice (brown stripe and grain rot). *Ann Phytopathol Soc Jpn* **21**, 46–47.

Greenberg, E. P. (1994). Quorum Sensing in Bacteria the LuxR-LuxI Family of Cell Density-Responsive Transcriptional Regulatorst **176**, 269–275.

Gunderson, F. & Cianciotto, N. (2013). The CRISPR-associated gene cas2 of Legionella pneumophila is required for intracellular infection of amoebae. *MBio* **4**, 1–11.

Ha, D.-G., Merritt, J. H., Hampton, T. H., Hodgkinson, J. T., Janecek, M., Spring, D. R., Welch, M. & O'Toole, G. a. **(2011).** 2-Heptyl-4-quinolone, a precursor of the Pseudomonas quinolone signal molecule, modulates swarming motility in Pseudomonas aeruginosa. *J Bacteriol* **193**, 6770–80.

Hager, A. J., Bolton, D. L., Pelletier, M. R., Brittnacher, M. J., Gallagher, L. a, Kaul, R., Skerrett, S. J., Miller, S. I. & Guina, T. (2006). Type IV pili-mediated secretion modulates Francisella virulence. *Mol Microbiol* **62**, 227–37.

Ham, J. H., Melanson, R. a & Rush, M. C. (2011, May). Burkholderia glumae: next major pathogen of rice? *Mol Plant Pathol*.

Hanzelka, B. L. & Greenberg, E. P. (1995). Evidence that the N-terminal region of the Vibrio fischeri LuxR protein constitutes an autoinducer-binding domain. *J Bacteriol* **177**, 815–7.

Hardcastle, T. J. & Kelly, K. a. **(2010).** baySeq: empirical Bayesian methods for identifying differential expression in sequence count data. *BMC Bioinformatics* **11**, 422.

Hauer, B., Friedrich, T., Nübling, C., Stürmer, R. & Lander, W. (2002). BUTINOL I ESTERASE.

Henrichsen, J. (1972). Bacterial Surface Translocation : a Survey and a Classification **36**, 478–503.

Hirakawa, H. & Tomita, H. (2013). Interference of bacterial cell-to-cell communication: a new concept of antimicrobial chemotherapy breaks antibiotic resistance. *Front Microbiol* **4**, 114.

Hoang, H. H., Gurich, N. & González, J. E. (2008). Regulation of motility by the ExpR/Sin quorum-sensing system in Sinorhizobium meliloti. *J Bacteriol* **190**, 861–71.

Van Houdt, R., Moons, P., Hueso Buj, M. & Michiels, C. W. (2006). N-acyl-L-homoserine lactone quorum sensing controls butanediol fermentation in Serratia plymuthica RVH1 and Serratia marcescens MG1. *J Bacteriol* **188**, 4570–2.

Van Houdt, R., Aertsen, A. & Michiels, C. W. (2007). Quorum-sensing-dependent switch to butanediol fermentation prevents lethal medium acidification in Aeromonas hydrophila AH-1N. *Res Microbiol* **158**, 379–385.

Huber, B., Riedel, K., Hentzer, M., Heydorn, a, Gotschlich, a, Givskov, M., Molin, S. & Eberl, L. (2001, September). The cep quorum-sensing system of Burkholderia cepacia H111 controls biofilm formation and swarming motility. *Microbiology*.

Ibuki, T., Uchida, Y., Hironaka, Y., Namba, K., Imada, K. & Minamino, T. (2013). Interaction between FliJ and FlhA, components of the bacterial flagellar type III export apparatus. *J Bacteriol* **195**, 466–73.

Jacobs, J. L., Fasi, A. C., Ramette, A., Smith, J. J., Hammerschmidt, R. & Sundin, G. W. (2008, May). Identification and onion pathogenicity of Burkholderia cepacia complex isolates from the onion rhizosphere and onion field soil. *Appl Environ Microbiol*.

Jang, M. S., Goo, E., An, J. H., Kim, J. & Hwang, I. (2014, January). Quorum Sensing Controls Flagellar Morphogenesis in Burkholderia glumae. *PLoS One*.

Jayaraman, A. & Wood, T. K. (2008). Bacterial quorum sensing: signals, circuits, and implications for biofilms and disease. *Annu Rev Biomed Eng* **10**, 145–67.

Jensen, V., Löns, D., Zaoui, C., Bredenbruch, F., Meissner, A., Dieterich, G., Münch, R. & Häussler, S. (2006). RhlR expression in Pseudomonas aeruginosa is modulated by the Pseudomonas quinolone signal via PhoB-dependent and -independent pathways. *J Bacteriol* **188**, 8601–6.

Jeong, Y., Kim, J., Kim, S., Kang, Y., Nagamatsu, T. & Hwang, I. (2003). Toxoflavin Produced by Burkholderia glumae Causing Rice Grain Rot Is Responsible for Inducing Bacterial Wilt in Many Field Crops. *Plant Dis* **87**, 890–895. Scientific Societies.

Jiang, Y., Camara, M., Chhabra, S. R., Hardie, K. R., Bycroft, B. W., Lazdunski, A., Salmond, G. P., Stewart, G. S. & Williams, P. (1998). In vitro biosynthesis of the Pseudomonas aeruginosa quorum-sensing signal molecule N-butanoyl-L-homoserine lactone. *Mol …* **28**, 193–203.

Jones, J., Chhabra, S. R., Downie, J. A. & Acteriol, J. B. (2002). raiIR Genes Are Part of a Quorum-Sensing Network Controlled by cinI and cinR in Rhizobium leguminosarum **184**, 1597–1606.

Kachlany, S. C., Planet, P. J., DeSalle, R., Fine, D. H., Figurski, D. H. & Kaplan, J. B. (2001). flp-1, the first representative of a new pilin gene subfamily, is required for non-specific adherence of Actinobacillus actinomycetemcomitans. *Mol Microbiol* **40**, 542–554.

Kang, Y.-S. & Park, W. (2010). Contribution of quorum-sensing system to hexadecane degradation and biofilm formation in Acinetobacter sp. strain DR1. *J Appl Microbiol* **109**, 1650–9.

Kaplan, H. B. & Greenberg, E. P. (1985). fischeri Luminescence System **163**, 1210–1214.

Karki, H. S., Shrestha, B. K., Han, J. W., Groth, D. E., Barphagha, I. K., Rush, M. C., Melanson, R. a, Kim, B. S. & Ham, J. H. (2012, January). Diversities in virulence, antifungal activity, pigmentation and DNA fingerprint among strains of Burkholderia glumae. *PLoS One*.

Kato, T., Morohoshi, T., Tsushima, S. & Ikeda, T. (2013). Phenotypic characterization of colony morphological mutants of Burkholderia glumae that emerged during subculture. *J Gen Plant* ….

Kelman, A. (1954). The relationship of pathogenicity of Pseudomonas solanacearum to colony appearance in a tetrazolium medium. *Phytopathology*.

Kessler, A., Schell, U., Sahr, T., Tiaden, A., Harrison, C., Buchrieser, C. & Hilbi, H. (2013). The Legionella pneumophila orphan sensor kinase LqsT regulates competence and pathogen-host interactions as a component of the LAI-1 circuit. *Environ Microbiol* **15**, 646–62.

Khajanchi, B. K., Sha, J., Kozlova, E. V, Erova, T. E., Suarez, G., Sierra, J. C., Popov, V. L., Horneman, A. J. & Chopra, A. K. (2009). N-acylhomoserine lactones involved in quorum sensing control the type VI secretion system, biofilm formation, protease production, and in vivo virulence in a clinical isolate of Aeromonas hydrophila. *Microbiology* **155**, 3518–31.

KHARB, R. P. S., LATHER, B. P. S. & DESWAL, D. P. (1994). Prediction of field emergence through heritability and genetic advance of vigour parameters. *Seed Sci Technol* **22**, 461–466. International Seed Testing Association.

El Khattabi, M., Van Gelder, P., Bitter, W. & Tommassen, J. (2000). Role of the lipase-specific foldase of Burkholderia glumae as a steric chaperone. *J Biol Chem* **275**, 26885–91.

Kim, H., Goo, E., Kang, Y., Kim, J. & Hwang, I. (2012). Regulation of universal stress protein genes by quorum sensing and RpoS in Burkholderia glumae. *J Bacteriol* **194**, 982–992.

Kim, J., Kim, J.-G., Kang, Y., Jang, J. Y., Jog, G. J., Lim, J. Y., Kim, S., Suga, H., Nagamatsu, T. & Hwang, I. (2004, November). Quorum sensing and the LysR-type transcriptional activator ToxR regulate toxoflavin biosynthesis and transport in Burkholderia glumae. *Mol Microbiol*.

Kim, J., Kang, Y., Choi, O., Jeong, Y., Jeong, J.-E., Lim, J. Y., Kim, M., Moon, J. S., Suga, H. & Hwang, I. (2007, April). Regulation of polar flagellum genes is mediated by quorum sensing and FlhDC in Burkholderia glumae. *Mol Microbiol*.

Kim, S., Park, J., Choi, O., Kim, J. & Seo, Y. (2014). Investigation of quorum sensing-dependent gene expression in Burkholderia gladioli BSR3 through RNA-seq analyses. *J Microbiol …* **24**, 1609–1621.

Kim, S., Park, J., Kim, J. H., Lee, J., Bang, B., Hwang, I. & Seo, Y.-S. (2013). RNAseq-based Transcriptome Analysis of Burkholderia glumae Quorum Sensing. *Plant Pathol J* **29**, 249–259.

Kinoshita, M., Hara, N., Imada, K., Namba, K. & Minamino, T. (2013). Interactions of bacterial flagellar chaperone-substrate complexes with FlhA contribute to co-ordinating assembly of the flagellar filament. *Mol Microbiol* **90**, 1249–61.

Kleerebezem, M. (2004). Quorum sensing control of lantibiotic production; nisin and subtilin autoregulate their own biosynthesis. *Peptides*.

Knapp, A., Voget, S., Gao, R., Zaburanny, N., Krysciak, D., Michael, B., Hauer, B., Streit, W. R., Müller, R. & other authors. **(2015).** Biotechnological potential of the lipase producing strain Burkholderia glumae PG1. *Appl Environ Microbiol* Submitted.

Kohler, T., Curty, L. K., Barja, F., van Delden, C. & Pechere, J.-C. (2000). Swarming of Pseudomonas aeruginosa Is Dependent on Cell-to-Cell Signaling and Requires Flagella and Pili. *J Bacteriol* **182**, 5990–5996.

Köhler, T., Curty, L. K., Barja, F., Delden, C. Van & Pechère, J. (2000). Swarming of Pseudomonas aeruginosa Is Dependent on Cell-to-Cell Signaling and Requires Flagella and Pili.

Kovach, M. E., Elzer, P. H., Hill, D. S., Robertson, G. T., Farris, M. a, Roop, R. M. & Peterson, K. M. (1995). Four new derivatives of the broad-host-range cloning vector pBBR1MCS, carrying different antibiotic-resistance cassettes. *Gene* **166**, 175–6.

Krysciak, D., Grote, J., Rodriguez Orbegoso, M., Utpatel, C., Förstner, K. U., Li, L., Schmeisser, C., Krishnan, H. B. & Streit, W. R. (2014). RNA Sequencing Analysis of the Broad-Host-Range Strain Sinorhizobium fredii NGR234 Identifies a Large Set of Genes Linked to Quorum Sensing-Dependent Regulation in the Background of a traI and ngrI Deletion Mutant. *Appl Environ Microbiol* **80**, 5655–71.

Langmead, B. & Salzberg, S. (2012). Fast gapped-read alignment with Bowtie 2. *Nat Methods* **9**, 357–359.

LaRock, C. N., Yu, J., Horswill, A. R., Parsek, M. R. & Minion, F. C. (2013). Transcriptome analysis of acyl-homoserine lactone-based quorum sensing regulation in Yersinia pestis [corrected]. *PLoS One* **8**, e62337.

Lassak, J., Henche, A.-L., Binnenkade, L. & Thormann, K. M. (2010, May). ArcS, the cognate sensor kinase in an atypical Arc system of Shewanella oneidensis MR-1. *Appl Environ Microbiol*.

Laue, B. E., Jiang, Y., Chhabra, S. R., Jacob, S., Stewart, G. S., Hardman, A., Downie, J. A., O'Gara, F. & Williams, P. (2000). The biocontrol strain Pseudomonas fluorescens F113 produces the Rhizobium small bacteriocin, N-(3-hydroxy-7-cis-tetradecenoyl)homoserine lactone, via HdtS, a putative novel N-acylhomoserine lactone synthase. *Microbiology* **146 (Pt 1**, 2469–80.

Lederberg, J. (1950). THE BETA-d-GALACTOSIDASE OF ESCHERICHIA COLI, STRAIN K-12 381–392.

Lee, J. & Zhang, L. (2014). The hierarchy quorum sensing network in Pseudomonas aeruginosa. *Protein Cell*.

Lewenza, S., Conway, B., Greenberg, E. P. & Sokol, P. A. (1999). Quorum sensing in Burkholderia cepacia: identification of the LuxRI homologs CepRI. *J Bacteriol* **181**, 748–56.

Li, C., Louise, C. J., Shi, W. & Adler, J. (1993). Adverse Conditions Which Cause Lack of Flagella in Escherichia coli **175**, 2229–2235.

Liu, H., Coulthurst, S. J., Pritchard, L., Hedley, P. E., Ravensdale, M., Humphris, S., Burr, T., Takle, G., Brurberg, M.-B. & other authors. **(2008).** Quorum sensing coordinates brute force and stealth modes of infection in the plant pathogen Pectobacterium atrosepticum. *PLoS Pathog* **4**, e1000093.

Louwen, R., Horst-Kreft, D., De Boer, a. G., Van Der Graaf, L., De Knegt, G., Hamersma, M., Heikema, a. P., Timms, a. R., Jacobs, B. C. & other authors. **(2013).** A novel link between Campylobacter jejuni bacteriophage defence, virulence and Guillain-Barr?? syndrome. *Eur J Clin Microbiol Infect Dis* **32**, 207–226.

Luo, J., Xie, G., Li, B. & Lihui, X. (2007). First Report of Burkholderia glumae Isolated from Symptomless Rice Seeds in China. *Plant Dis* **91**, 1363. Scientific Societies.

Luo, Z. Q., Clemente, T. E. & Farrand, S. K. (2001). Construction of a derivative of Agrobacterium tumefaciens C58 that does not mutate to tetracycline resistance. *Mol Plant Microbe Interact* **14**, 98–103.

Lupp, C., Urbanowski, M., Greenberg, E. P. & Ruby, E. G. (2003). The Vibrio fischeri quorum-sensing systems ain and lux sequentially induce luminescence gene expression and are important for persistence in the squid host **50**, 319–331.

Lutter, E., Lewenza, S., Dennis, J. J., Visser, M. B. & Sokol, P. A. (2001). Distribution of quorum-sensing genes in the Burkholderia cepacia complex. *Infect Immun* **69**, 4661–4666.

Majerczyk, C., Brittnacher, M., Jacobs, M., Armour, C. D., Radey, M., Schneider, E., Phattarasokul, S., Bunt, R. & Greenberg, E. P. (2014a). Global Analysis of the Burkholderia thailandensis quorum-sensing-controlled regulon. *J Bacteriol* **196**, 1412–24.

Majerczyk, C. D., Brittnacher, M. J., Jacobs, M. a, Armour, C. D., Radey, M. C., Bunt, R., Hayden, H. S., Bydalek, R. & Greenberg, E. P. (2014b). A Cross-species Comparison of the Burkholderia pseudomallei, Burkholderia thailandensis, and Burkholderia mallei Quorum Sensing Regulons. *J Bacteriol*.

Makarova, K. S., Haft, D. H., Barrangou, R., Brouns, S. J. J., Charpentier, E., Horvath, P., Moineau, S., Mojica, F. J. M., Wolf, Y. I. & other authors. **(2011).** Evolution and classification of the CRISPR-Cas systems. *Nat Rev Microbiol* **9**, 467–77. Nature Publishing Group.

Malott, R. J. & Sokol, P. a. **(2007).** Expression of the bviIR and cepIR quorum-sensing systems of Burkholderia vietnamiensis. *J Bacteriol* **189**, 3006–16.

Malott, R. J., Baldwin, A., Mahenthiralingam, E. & Sokol, P. A. (2005). Characterization of the cciIR quorum-sensing system in Burkholderia cenocepacia. *Infect Immun* **73**, 4982–92.

Manefield, M., Rasmussen, T. B., Henzter, M., Andersen, J. B., Steinberg, P., Kjelleberg, S. & Givskov, M. (2002). Halogenated furanones inhibit quorum sensing through accelerated LuxR turnover. *Microbiology* **148**, 1119–27.

Master, E. & Mohn, W. (1998). Psychrotolerant bacteria isolated from arctic soil that degrade polychlorinated biphenyls at low temperatures. *Appl Environ Microbiol* **64**, 4823–9.

Mavrodi, D. V, Blankenfeldt, W. & Thomashow, L. S. (2006). Phenazine compounds in fluorescent Pseudomonas spp. biosynthesis and regulation. *Annu Rev Phytopathol* **44**, 417–45.

McCarter, L. (2006). Regulation of flagella. *Curr Opin Microbiol*.

Mcclean, K. H., Winson, M. K., Fish, L., Taylor, A., Chhabra, S. R., Camara, M., Daykin, M., John, H., Swift, S. & other authors. **(1997).** Quorum sensing and Chrornobacteriurn violaceurn : exploitation of violacein production and inhibition for the detection of N-acyl homoserine lactones.

McGowan, S. J., Barnard, A. M. L., Bosgelmez, G., Sebaihia, M., Simpson, N. J. L., Thomson, N. R., Todd, D. E., Welch, M., Whitehead, N. a & Salmond, G. P. C. (2005). Carbapenem antibiotic biosynthesis in Erwinia carotovora is regulated by physiological and genetic factors modulating the quorum sensing-dependent control pathway. *Mol Microbiol* **55**, 526–45.

McKenney, D., Brown, K. E. & Allison, D. G. (1995). Influence of Pseudomonas aeruginosa exoproducts on virulence factor production in Burkholderia cepacia: evidence of interspecies communication. *J Bacteriol* **177**, 6989–92.

Medina, G., Jua, K., Valderrama, B. & Sobero, G. (2003). Mechanism of Pseudomonas aeruginosa RhlR Transcriptional Regulation of the rhlAB Promoter **185**, 5976–5983.

Miller, M. B., Skorupski, K., Lenz, D. H., Taylor, R. K. & Bassler, B. L. (2002). Parallel quorum sensing systems converge to regulate virulence in Vibrio cholerae. *Cell* **110**, 303–314.

Minamino, T., Chu, R., Yamaguchi, S. & Macnab, M. (2000). Role of FliJ in Flagellar Protein Export in Salmonella Role of FliJ in Flagellar Protein Export in Salmonella **182**.

Minogue, T. D., Carlier, A. L., Koutsoudis, M. D. & Von Bodman, S. B. (2005). The cell density-dependent expression of stewartan exopolysaccharide in Pantoea stewartii ssp. stewartii is a function of EsaR-mediated repression of the rcsA gene. *Mol Microbiol* **56**, 189–203.

Miyata, S. T., Bachmann, V. & Pukatzki, S. (2013). Type VI secretion system regulation as a consequence of evolutionary pressure. *J Med Microbiol* **62**, 663–76.

Moré, M., Finger, L., Stryker, J. & Fuqua, C. (1996). Enzymatic synthesis of a quorum-sensing autoinducer through use of defined substrates. *Science (80-)*.

Mougous, J., Cuff, M., Raunser, S. & Shen, A. (2006). A virulence locus of Pseudomonas aeruginosa encodes a protein secretion apparatus. *Science (80-)* **312**, 1526–1530.

Nandakumar, R., Rush, M. C. & Correa, F. (2007). Association of Burkholderia glumae and B. gladioli with Panicle Blight Symptoms on Rice in Panama. *Plant Dis* **91**, 767–767. Scientific Societies.

Nawani, N., Khurana, J. & Kaur, J. (2006). A thermostable lipolytic enzyme from a thermophilic Bacillus sp.: purification and characterization. *Mol Cell Biochem* **290**, 17–22.

Ng, W.-L. & Bassler, B. L. (2009). Bacterial quorum-sensing network architectures. *Annu Rev Genet* **43**, 197–222.

Nijvipakul, S., Wongratana, J., Suadee, C., Entsch, B., Ballou, D. P. & Chaiyen, P. (2008). LuxG is a functioning flavin reductase for bacterial luminescence. *J Bacteriol* **190**, 1531–8.

Nimtz, M., Domke, T., Wray, V. & Ha, S. (1998). Purification and Characterization of a Cytotoxic Exolipid of Burkholderia pseudomallei **66**, 1588–1593.

Nookaew, I., Papini, M., Pornputtapong, N., Scalcinati, G., Fagerberg, L., Uhlén, M. & Nielsen, J. (2012). A comprehensive comparison of RNA-Seq-based transcriptome analysis from reads to differential gene expression and cross-comparison with microarrays: a case study in Saccharomyces cerevisiae. *Nucleic Acids Res* **40**, 10084–97.

O'Grady, E. P., Viteri, D. F., Malott, R. J. & Sokol, P. a. **(2009).** Reciprocal regulation by the CepIR and CciIR quorum sensing systems in Burkholderia cenocepacia. *BMC Genomics* **10**, 441.

O'Loughlin, C. T., Miller, L. C., Siryaporn, A., Drescher, K., Semmelhack, M. F. & Bassler, B. L. (2013). A quorum-sensing inhibitor blocks Pseudomonas aeruginosa virulence and biofilm formation. *Proc Natl Acad Sci U S A* **110**, 17981–6.

Ochsner, U. R. S. A., Reiser, J., Fiechter, A. & Witholt, B. (1995). Production of Pseudomonas aeruginosa Rhamnolipid Biosurfactants in Heterologous Hosts **61**, 3503–3506.

Olvera, C., Goldberg, J. B., Sa, R. & Sobero, G. (1999). The Pseudomonas aeruginosa algC gene product participates in rhamnolipid biosynthesis **179**, 85–90.

Oomey, M. I. K. (1998). Suppression of an absolute defect in Type IV pilus biogenesis by loss-of-function mutations in pilT , a twitching motility gene in Neisseria gonorrhoeae **95**, 14973–14978.

Overhage, J., Lewenza, S., Marr, A. K. & Hancock, R. E. W. (2007). Identification of genes involved in swarming motility using a Pseudomonas aeruginosa PAO1 mini-Tn5-lux mutant library. *J Bacteriol* **189**, 2164–9.

Pai, A., Tanouchi, Y. & You, L. (2012). Optimality and robustness in quorum sensing (QS)-mediated regulation of a costly public good enzyme. *Proc Natl Acad Sci U S A* **109**, 19810–5.

Palmer, K. L. & Whiteley, M. (2011). DMS3-42: the secret to CRISPR-dependent biofilm inhibition in Pseudomonas aeruginosa. *J Bacteriol* **193**, 3431–2.

Pappas, K. M., Weingart, C. L. & Winans, S. C. (2004). Chemical communication in proteobacteria: biochemical and structural studies of signal synthases and receptors required for intercellular signalling. *Mol Microbiol* **53**, 755–769.

Parsek, M. R., Val, D. L., Hanzelka, B. L., Cronan, J. E. & Greenberg, E. P. (1999). Acyl homoserine-lactone quorum-sensing signal generation. *Proc Natl Acad Sci U S A* **96**, 4360–5.

Pearson, J. P., Pesci, E. C. & Iglewski, B. H. (1997). Roles of Pseudomonas aeruginosa las and rhl quorum-sensing systems in control of elastase and rhamnolipid biosynthesis genes. *J Bacteriol* **179**, 5756–5767.

Pereira, C. S., Thompson, J. a & Xavier, K. B. (2013). AI-2-mediated signalling in bacteria. *FEMS Microbiol Rev* **37**, 156–81.

Pfaffl, M. W., Horgan, G. W. & Dempfle, L. (2002). Relative expression software tool (REST) for group-wise comparison and statistical analysis of relative expression results in real-time PCR. *Nucleic Acids Res* **30**, e36.

Pilatz, S., Breitbach, K., Hein, N., Fehlhaber, B., Schulze, J., Brenneke, B., Eberl, L. & Steinmetz, I. (2006). Identification of Burkholderia pseudomallei genes required for the intracellular life cycle and in vivo virulence. *Infect Immun* **74**, 3576–86.

Planet, P. J., Kachlany, S. C., DeSalle, R. & Figurski, D. H. (2001). Phylogeny of genes for secretion NTPases: identification of the widespread tadA subfamily and development of a diagnostic key for gene classification. *Proc Natl Acad Sci U S A* **98**, 2503–8.

Pukatzki, S., Ma, A. T., Sturtevant, D., Krastins, B., Sarracino, D., Nelson, W. C., Heidelberg, J. F. & Mekalanos, J. J. (2006). Identification of a conserved bacterial protein secretion system in Vibrio cholerae using the Dictyostelium host model system. *Proc Natl Acad Sci U S A* **103**, 1528–33.

Qian, G., Zhou, Y., Zhao, Y., Song, Z., Wang, S., Fan, J., Hu, B., Venturi, V. & Liu, F. (2013). Proteomic analysis reveals novel extracellular virulence-associated proteins and functions regulated by the diffusible signal factor (DSF) in Xanthomonas oryzae pv. oryzicola. *J Proteome Res* **12**, 3327–41.

Quadri, L. E. N. (2002). Regulation of antimicrobial peptide production by autoinducer-mediated quorum sensing in lactic acid bacteria. *Antonie Van Leeuwenhoek* **82**, 133–45.

Quiñones, B., Miller, W. G., Bates, A. H. & Mandrell, R. E. (2009). Autoinducer-2 production in Campylobacter jejuni contributes to chicken colonization. *Appl Environ Microbiol* **75**, 281–5.

Rader, B. a, Campagna, S. R., Semmelhack, M. F., Bassler, B. L. & Guillemin, K. (2007). The quorum-sensing molecule autoinducer 2 regulates motility and flagellar morphogenesis in Helicobacter pylori. *J Bacteriol* **189**, 6109–17.

Rahim, R., Ochsner, U. A., Olvera, C., Graninger, M., Messner, P., Lam, J. S. & Sobero, G. (2001). Cloning and functional characterization of the Pseudomonas aeruginosa rhlC gene that encodes rhamnosyltransferase 2 , an enzyme responsible for di-rhamnolipid biosynthesis **40**, 708–718.

Rajeshwari, R., Jha, G. & Sonti, R. V. **(2005).** Role of an In Planta – Expressed Xylanase of Xanthomonas oryzae pv . oryzae in Promoting Virulence on Rice **18**, 830–837.

Ravn, L., Christensen, a B., Molin, S., Givskov, M. & Gram, L. (2001). Methods for detecting acylated homoserine lactones produced by Gram-negative bacteria and their application in studies of AHL-production kinetics. *J Microbiol Methods* **44**, 239–51.

Richter, C. & Fineran, P. C. (2013). The subtype I-F CRISPR-Cas system influences pathogenicity island retention in Pectobacterium atrosepticum via crRNA generation and Csy complex formation. *Biochem Soc Trans* **41**, 1468–74.

Richter, C., Dy, R. L., McKenzie, R. E., Watson, B. N. J., Taylor, C., Chang, J. T., McNeil, M. B., Staals, R. H. J. & Fineran, P. C. (2014). Priming in the Type I-F CRISPR-Cas system triggers strand-independent spacer acquisition, bi-directionally from the primed protospacer. *Nucleic Acids Res* **42**, 8516–26.

Rivas, M., Seeger, M., Jedlicki, E. & Holmes, D. S. (2007). Second acyl homoserine lactone production system in the extreme acidophile Acidithiobacillus ferrooxidans. *Appl Environ Microbiol* **73**, 3225–3231.

Ruijter, J. M., Ramakers, C., Hoogaars, W. M. H., Karlen, Y., Bakker, O., van den Hoff, M. J. B. & Moorman, a F. M. (2009). Amplification efficiency: linking baseline and bias in the analysis of quantitative PCR data. *Nucleic Acids Res* **37**, e45.

Rutherford, S. T. & Bassler, B. L. (2012). Bacterial quorum sensing: its role in virulence and possibilities for its control. *Cold Spring Harb Perspect Med* **2**.

Sambrook, J. & Russell, D. W. (2001). Molecular Cloning: A Laboratory Manual, Third Edition. *Cold Spring Harb Lab Press* **1-3**.

Sampson, T. R., Saroj, S. D., Llewellyn, A. C., Tzeng, Y.-L. & Weiss, D. S. (2013). A CRISPR/Cas system mediates bacterial innate immune evasion and virulence. *Nature* **497**, 254–7. Nature Publishing Group.

Sanger, F. & Nicklen, S. (1977). DNA sequencing with chain-terminating **74**, 5463–5467.

Schaefer, a L., Val, D. L., Hanzelka, B. L., Cronan, J. E. & Greenberg, E. P. (1996). Generation of cell-to-cell signals in quorum sensing: acyl homoserine lactone synthase activity of a purified Vibrio fischeri LuxI protein. *Proc Natl Acad Sci U S A* **93**, 9505–9.

Schell, M. a, Ulrich, R. L., Ribot, W. J., Brueggemann, E. E., Hines, H. B., Chen, D., Lipscomb, L., Kim, H. S., Mrázek, J. & other authors. **(2007).** Type VI secretion is a major virulence determinant in Burkholderia mallei. *Mol Microbiol* **64**, 1466–85.

Schipper, C. (2009). -Biofilm Strategien aus Metagenom-Isolierung und Charakterisierung Quorum Sensing inhibierender Proteine aus Genbanken direkt isolierter Umwelt-DNA.

Schmittgen, T. D. & Livak, K. J. (2008). Analyzing real-time PCR data by the comparative CT method. *Nat Protoc* **3**, 1101–1108.

Schwarz, S., West, T. E., Boyer, F., Chiang, W.-C., Carl, M. a, Hood, R. D., Rohmer, L., Tolker-Nielsen, T., Skerrett, S. J. & Mougous, J. D. (2010). Burkholderia type VI secretion systems have distinct roles in eukaryotic and bacterial cell interactions. *PLoS Pathog* **6**, e1001068.

Schwarz, S., Singh, P., Robertson, J. D., LeRoux, M., Skerrett, S. J., Goodlett, D. R., West, T. E. & Mougous, J. D. (2014). VgrG-5 is a Burkholderia type VI secretion system-exported protein required for multinucleated giant cell formation and virulence. *Infect Immun* **82**, 1445–52.

Scott, R. J., Lian, L.-Y., Muharram, S. H., Cockayne, A., Wood, S. J., Bycroft, B. W., Williams, P. & Chan, W. C. (2003). Side-chain-to-tail thiolactone peptide inhibitors of the staphylococcal quorum-sensing system. *Bioorg Med Chem Lett* **13**, 2449–2453.

Shaw, P. D., Ping, G., Daly, S. L., Cha, C., Cronan, J. E., Rinehart, K. L. & Farrand, S. K. (1997). Detecting and characterizing N-acyl-homoserine lactone signal molecules by thin-layer chromatography. *Proc Natl Acad Sci U S A* **94**, 6036–41.

Shen, B., Du, L., Sanchez, C., Edwards, D. J., Chen, M. & Murrell, J. M. (2001). The biosynthetic gene cluster for the anticancer drug bleomycin from Streptomyces verticillus ATCC15003 as a model for hybrid peptide-polyketide natural product biosynthesis. *J Ind Microbiol Biotechnol* **27**, 378–385.

Shi, W., Zhou, Y., Wild, J., Adler, J. & Gross, C. A. (1992). DnaK , DnaJ , and GrpE Are Required for Flagellum Synthesis in Escherichia coli **174**, 6256–6263.

Shi, W., Li, C., Louise, C. & Adler, J. (1993). Mechanism of adverse conditions causing lack of flagella in Escherichia coli. *J Bacteriol* **175**, 2236–2240.

Shingu, Y. & Yoneyama, K. (2004). Essential regulator gene tox R for toxoflavin biosynthesis of Burkholderia glumae. *J Gen Plant Pathol* **70**, 108–114. Springer-Verlag.

Shyntum, D. Y., Venter, S. N., Moleleki, L. N., Toth, I. & Coutinho, T. A. (2014). Comparative genomics of type VI secretion systems in strains of Pantoea ananatis from different environments. *BMC Genomics* **15**, 163. BMC Genomics.

Solis, R., Bertani, I., Degrassi, G., Devescovi, G. & Venturi, V. (2006). Involvement of quorum sensing and RpoS in rice seedling blight caused by Burkholderia plantarii. *FEMS Microbiol Lett* **259**, 106–112. Blackwell Publishing Ltd.

Solovyev, V. & Salamov, A. (2011). Automatic Annotation of Microbial Genomes and Metagenomic Sequences. In *Metagenomics its Appl Agric Biomed Environ Stud (Ed RW Li)*, pp. 61–78.

Soutourina, O. a & Bertin, P. N. (2003). Regulation cascade of flagellar expression in Gram-negative bacteria. *FEMS Microbiol Rev* **27**, 505–523.

Sperandio, V., Torres, A. G. & Kaper, J. B. (2002). Quorum sensing Escherichia coli regulators B and C (QseBC): a novel two-component regulatory system involved in the regulation of flagella and motility by quorum sensing in E . coli **43**, 809–821.

Starnbach, M. & Lory, S. (1992). The filA (rpoF) gene of Pseudomonas aeruginosa encodes an alternative sigma factor required for flagellin synthesis. *Mol Microbiol* **6**.

Stevens, a M., Dolan, K. M. & Greenberg, E. P. (1994). Synergistic binding of the Vibrio fischeri LuxR transcriptional activator domain and RNA polymerase to the lux promoter region. *Proc Natl Acad Sci U S A* **91**, 12619–23.

Sturme, M. H. J., Kleerebezem, M., Nakayama, J., Akkermans, A. D. L., Vaugha, E. E. & de Vos, W. M. (2002). Cell to cell communication by autoinducing peptides in gram-positive bacteria. *Antonie Van Leeuwenhoek* **81**, 233–43.

Suárez-Moreno, Z. R., Devescovi, G., Myers, M., Hallack, L., Mendonça-Previato, L., Caballero-Mellado, J. & Venturi, V. (2010, July). Commonalities and differences in regulation of N-acyl homoserine lactone quorum sensing in the beneficial plant-associated burkholderia species cluster. *Appl Environ Microbiol*.

Suárez-Moreno, Z. R., Caballero-Mellado, J., Coutinho, B. G., Mendonça-Previato, L., James, E. K. & Venturi, V. (2012). Common features of environmental and potentially beneficial plant-associated Burkholderia. *Microb Ecol* **63**, 249–66.

Sun, H., Zusman, D. R. & Shi, W. (2000). Type IV pilus of Myxococcus xanthus is a motility apparatus controlled by the frz chemosensory system. *Curr Biol* **10**, 1143–1146.

Suzuki, F., Sawada, H., Azegami, K. & Tsuchiya, K. (2004). Molecular characterization of the tox operon involved in toxoflavin biosynthesis of Burkholderia glumae. *J Gen Plant …*.

Taguchi, F. & Ichinose, Y. (2011). Role of Type IV Pili in Virulence of Pseudomonas syringae pv . tabaci 6605 : Correlation of Motility , Multidrug Resistance , and HR-Inducing Activity on a Nonhost Plant **24**, 1001–1011.

Tavares, L. F. D., Silva, P. M., Junqueira, M., Mariano, D. C. O., Nogueira, F. C. S., Domont, G. B., Freire, D. M. G. & Neves, B. C. (2013). Characterization of rhamnolipids produced by wild-type and engineered Burkholderia kururiensis. *Appl Microbiol Biotechnol* **97**, 1909–21.

Tiaden, A., Spirig, T., Sahr, T., Wälti, M. a, Boucke, K., Buchrieser, C. & Hilbi, H. (2010a). The autoinducer synthase LqsA and putative sensor kinase LqsS regulate phagocyte interactions, extracellular filaments and a genomic island of Legionella pneumophila. *Environ Microbiol* **12**, 1243–59.

Tiaden, A., Spirig, T., Sahr, T., Wälti, M. a, Boucke, K., Buchrieser, C. & Hilbi, H. (2010b). The autoinducer synthase LqsA and putative sensor kinase LqsS regulate phagocyte interactions, extracellular filaments and a genomic island of Legionella pneumophila. *Environ Microbiol* **12**, 1243–59.

Tomich, M., Fine, D. H. & Figurski, D. H. (2006). The TadV protein of Actinobacillus actinomycetemcomitans is a novel aspartic acid prepilin peptidase required for maturation of the Flp1 pilin and TadE and TadF pseudopilins. *J Bacteriol* **188**, 6899–914.

Tomich, M., Planet, P. J. & Figurski, D. H. (2007). The tad locus: postcards from the widespread colonization island. *Nat Rev Microbiol* **5**, 363–75.

Trung H.M., Van N.V., Vien N.V., L. D. T. and L. M. (1993). Occurrenceof rice grain rot disease in Vietnam. *Int Rice Res Notes* **18**, 30.

Udine, C., Brackman, G., Bazzini, S., Buroni, S., Van Acker, H., Pasca, M. R., Riccardi, G. & Coenye, T. (2013, January). Phenotypic and genotypic characterisation of Burkholderia cenocepacia J2315 mutants affected in homoserine lactone and diffusible signal factor-based quorum sensing systems suggests interplay between both types of systems. *PLoS One*.

Ulrich, R. L., Hines, H. B., Parthasarathy, N. & Jeddeloh, J. a. **(2004, July).** Mutational analysis and biochemical characterization of the Burkholderia thailandensis DW503 quorum-sensing network. *J Bacteriol*.

Untergasser, A., Cutcutache, I., Koressaar, T., Ye, J., Faircloth, B. C., Remm, M. & Rozen, S. G. (2012). Primer3--new capabilities and interfaces. *Nucleic Acids Res* **40**, e115.

Vanlaere, E., Baldwin, A., Gevers, D., Henry, D., De Brandt, E., LiPuma, J. J., Mahenthiralingam, E., Speert, D. P., Dowson, C. & Vandamme, P. (2009). Taxon K, a complex within the Burkholderia cepacia complex, comprises at least two novel species, Burkholderia contaminans sp. nov. and Burkholderia lata sp. nov. *Int J Syst Evol Microbiol* **59**, 102–11.

Venturi, V. (2006). Regulation of quorum sensing in Pseudomonas. *FEMS Microbiol Rev* **30**, 274–91.

Voget, S., Knapp, A., Poehlein, A., Vollstedt, C., Streit, W., Daniel, R. & Jaeger, K.-E. (2015). Complete genome sequence of the lipase producing strain Burkholderia glumae PG1. *J Biotechnol* **204**, 3–4. Elsevier B.V.

Voigt, C. a, Schäfer, W. & Salomon, S. (2005). A secreted lipase of Fusarium graminearum is a virulence factor required for infection of cereals. *Plant J* **42**, 364–75.

Wall, D. & Kaiser, D. (1999). Type IV pili and cell motility. *Mol Microbiol* **32**, 1–10.

Wang, Z., Lin, B., Mostaghim, A., Rubin, R. a, Glaser, E. R., Mittraparp-Arthorn, P., Thompson, J. R., Vuddhakul, V. & Vora, G. J. (2013). Vibrio campbellii hmgA-mediated pyomelanization impairs quorum sensing, virulence, and cellular fitness. *Front Microbiol* **4**, 379.

Waters, C. M. & Bassler, B. L. (2005). Quorum sensing: cell-to-cell communication in bacteria. *Annu Rev Cell Dev Biol* **21**, 319–46.

Waters, C. M. & Bassler, B. L. (2006). The Vibrio harveyi quorum-sensing system uses shared regulatory components to discriminate between multiple autoinducers. *Genes Dev* **20**, 2754–67.

Watson, W. T., Minogue, T. D., Val, D. L., Von Bodman, S. B. & Churchill, M. E. A. (2002). Structural basis and specificity of acyl-homoserine mactone signal production in bacterial quorum sensing. *Mol Cell* **9**, 685–694.

Weinberg, J. B., Alexander, B. D., Majure, J. M., Williams, L. W., Kim, J. Y., Vandamme, P. & LiPuma, J. J. (2007). Burkholderia glumae infection in an infant with chronic granulomatous disease. *J Clin Microbiol* **45**, 662–5.

Westra, E. R., Buckling, A. & Fineran, P. C. (2014). CRISPR-Cas systems: beyond adaptive immunity. *Nat Rev Microbiol* **12**, 317–26. Nature Publishing Group.

Williams, P. (2007). Quorum sensing, communication and cross-kingdom signalling in the bacterial world. *Microbiology* **153**, 3923–38.

Williams, P., Camara, M., Hardman, A., Swift, S., Hope, V. J., Winzer, K., Middleton, B., Pritchard, D. I., Bycroft, B. W. & Milton, D. (2000). Quorum Sensing and the Population-Dependent Control of Virulence. *Act Bact Pathog Vivo - Based Contrib to a R Soc Discuss Meet* 283–316. London: Imperial College Press.

Williamson, N. R., Fineran, P. C., Ogawa, W., Woodley, L. R. & Salmond, G. P. C. (2008). Integrated regulation involving quorum sensing, a two-component system, a GGDEF/EAL domain protein and a post-transcriptional regulator controls swarming and RhlA-dependent surfactant biosynthesis in Serratia. *Environ Microbiol* **10**, 1202–17.

Xavier, K. B. & Bassler, B. L. (2005). Regulation of uptake and processing of the quorum-sensing autoinducer AI-2 in Escherichia coli. *J Bacteriol* **187**, 238–48.

Xavier, K. B., Miller, S. T., Lu, W., Kim, J. H., Rabinowitz, J., Pelczer, I., Semmelhack, M. F. & Bassler, B. L. (2007). Phosphorylation and processing of the quorum-sensing molecule autoinducer-2 in enteric bacteria. *ACS Chem Biol* **2**, 128–136.

Yabuuchi, E., Kosako, Y. & Oyaizu, H. (1992). of seven species of the genus Pseudomonas homology group II to the new genus, with the type species Burkholderia cepacia (Palleroni and Holmes 1981) comb. nov. *Microbiol …* **36**, 1251–1275.

Yang, Q. & Defoirdt, T. (2014). Quorum sensing positively regulates flagellar motility in pathogenic Vibrio harveyi. *Environ Microbiol*.

Yao, F., Zhou, H. & Lessie, T. G. (2002). Characterization of N-acyl homoserine lactone overproducing mutants of Burkholderia multivorans ATCC 17616. *FEMS Microbiol Lett* **206**, 201–207. Blackwell Publishing Ltd.

Yong, Y. C. & Zhong, J. J. (2010). N-Acylated homoserine lactone production and involvement in the biodegradation of aromatics by an environmental isolate of Pseudomonas aeruginosa. *Process Biochem* **45**, 1944–1948.

Zegans, M. E., Wagner, J. C., Cady, K. C., Murphy, D. M., Hammond, J. H. & O'Toole, G. a. **(2009).** Interaction between bacteriophage DMS3 and host CRISPR region inhibits group behaviors of Pseudomonas aeruginosa. *J Bacteriol* **191**, 210–9.

Zeigler, R. S. & Alvarez, E. (1989). Grain discoloration of rice caused by Pseudomonas glumae in Latin America. *Plant Dis* **73**, 368.

Zhang, R., Pappas, K. M., Pappas, T., Brace, J. L., Miller, P. C., Oulmassov, T., Molyneaux, J. M., Anderson, J. C., Bashkin, J. K. & other authors. **(2002).** Structure of a bacterial quorum-sensing transcription factor complexed with pheromone and DNA. *Nature* **417**, 971–4.

Zheng, J. & Leung, K. Y. (2007). Dissection of a type VI secretion system in Edwardsiella tarda. *Mol Microbiol* **66**, 1192–206.

Zhou, H., Yao, F., Roberts, D. P. & Lessie, T. G. (2003). AHL-Deficient Mutants of Burkholderia ambifaria BC-F Have Decreased Antifungal Activity. *Curr Microbiol* **47**, 174–179.

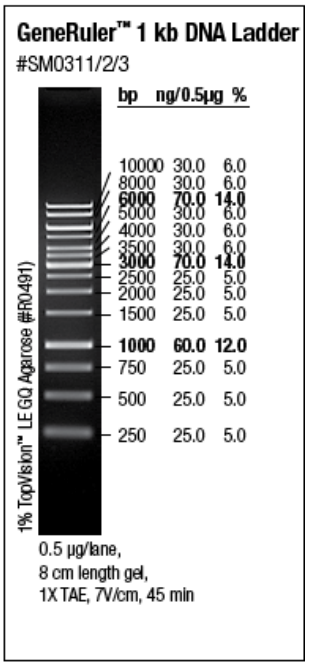
Zhou, X. G. (2013). First Report of Bacterial Panicle Blight of Rice Caused by Burkholderia glumae in South Africa. *Plant Dis* **98**, 566. Scientific Societies.

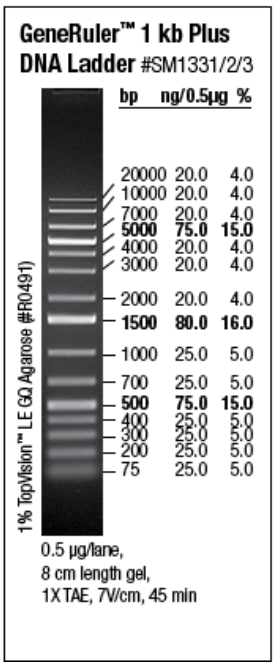
Zhu, J. & Winans, S. C. (2001). The quorum-sensing transcriptional regulator TraR requires its cognate signaling ligand for protein folding, protease resistance, and dimerization. *Proc Natl Acad Sci U S A* **98**, 1507–12.

Zhu, J., Beaber, J. W., Moré, M. I., Fuqua, C., Eberhard, A. & Winans, S. C. (1998). Analogs of the autoinducer 3-oxooctanoyl-homoserine lactone strongly inhibit activity of the TraR protein of Agrobacterium tumefaciens. *J Bacteriol* **180**, 5398–5405.

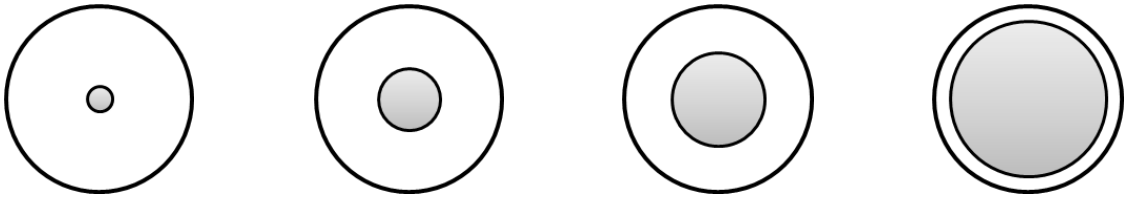
VI. Appendix

1. GeneRuler (Fermentas, St-Leon-Rot, Germany)





2. Evaluation scheme for swarming or swimming motility



No swarming or swimming | Minimal swarming or swimming | Moderate swarming or swimming | Strong swarming or swimming

3. Promoter sequences of *bgal1-3* genes in *B. glumae* PG1

bgal1: AACAG**TTACCT**GTCCTCGGGGCGCA**CGTTGTAAT**GCGCC
-35 box -10 box

bgal2: CGAGT**TTTCCC**CGCGCGGCCCGGC**GTGTGTAAT**CGGCCT
-35 box -10 box

bgal3: AACC**TTGCCA**GTTGCAAACGCCGACGGT**TGCGATAGT**TTT
-35 box -10 box

Figure 35. Promoter sequence analysis of *bgal1-3* genes in *B. glumae* PG1. This analysis of promoter sequences was performed by using the web service of BPROM (Solovyev & Salamov, 2011) (http://www.softberry.com/berry.phtml?topic=bprom&group=programs&subgroup=gfindb).

4. All QS-regulated genes by *bgaIR1-3* QS systems in *B. glumae* PG1

Table 18. QS-regulated genes for BGPG2-4 vs. BGPG1.

Locus_tag	Predicted function	Fold change		
		BGPG2	BGPG3	BGPG4
BGL_1c00060	catalase	2.1-		
BGL_1c00150	flagellar biosynthetic protein FliQ		2.4+	2.8+
BGL_1c00160	flagellar biosynthetic protein FliP		3.1+	3.6+
BGL_1c00190	flagellar motor switch protein FliM		2.2+	2.7+
BGL_1c00200	flagellar basal body-associated protein FliL	2.0+	3.4+	5.6+
BGL_1c00490	pterin-4-alpha-carbinolamine dehydratase	2.0+		
BGL_1c00500	phenylalanine-4-hydoxylase PhhA		3.2-	3.3-
BGL_1c00800	aminotransferase class I and II	2.0-	2.1-	2.6-
BGL_1c00810	lysine-arginine-ornithine-binding periplasmic protein ArgT	2.2-	2.8-	4.0-
BGL_1c00940	thiamine pyrophosphate enzyme domain protein TPP-binding			2.5-
BGL_1c01420	3-oxoacyl-(acyl carrier protein) synthase III FabH		2.8+	3.4+
BGL_1c01430	aminotransferase, DegT/DnrJ/EryC1/StrS family		2.1+	2.8+
BGL_1c01440	TPR domain protein		2.6+	3.4+
BGL_1c01520	histone-like nucleoid-structuring (H-NS) protein	8.7-		
BGL_1c01540	flagellar transcriptional activator FlhD		2.5+	2.7+
BGL_1c01550	flagellar transcriptional activator FlhC			2.0+
BGL_1c01560	chemotaxis protein MotA			2.1+
BGL_1c01700	flagellar biosynthetic protein FlhB		3.4+	4.7+
BGL_1c01710	flagellar biosynthesis protein FlhA		3.1+	4.4+
BGL_1c01720	flagellar biosynthesis protein FlhF		2.5+	3.2+
BGL_1c01730	flagellar biosynthesis protein FlhG			2.3+
BGL_1c01740	RNA polymerase, sigma factor FliA		2.2+	2.9+
BGL_1c01870	hypothetical protein			2.3-
BGL_1c01890	rare lipoprotein A	3.4+		
BGL_1c02030	hypothetical protein		3.2+	2.7+
BGL_1c02100	hypothetical protein		2.1+	
BGL_1c02640	ribosomal protein L14			3.1+
BGL_1c03130	thiamine-phosphate diphosphorylase	2.0-		2.5-
BGL_1c03410	ubiquinol-cytochrome c reductase, iron-sulphur subunit	2.3-		
BGL_1c03420	cytochrome b	2.8-		
BGL_1c03540	type VI secretion system inner membrane protein, DotU family	2.1-		
BGL_1c03550	type VI secretion system protein, VC_A0114 family	2.2-		
BGL_1c03560	type VI secretion system, lipoprotein VCA0113 family	2.4-		
BGL_1c03570	TPR domain protein	3.1-		
BGL_1c03580	type VI secretion system protein, VC_A0107 family	3.1-		
BGL_1c03590	type VI secretion protein, EvpB/VC_A0108 family	3.1-		

Locus_tag	Predicted function	Fold change		
		BGPG2	BGPG3	BGPG4
BGL_1c03600	type VI secretion system effector, Hcp family	3.1-		
BGL_1c03610	type VI secretion system lysozyme-like protein HsiF	2.4-		
BGL_1c03620	type VI secretion system protein, VC_A0110 family	2.5-		
BGL_1c03630	type VI secretion system protein, VC_A0111 family	2.4-		
BGL_1c03640	type VI secretion system ATPase, ClpV1 family	2.6-		
BGL_1c03650	type VI secretion-associated protein, ImpA family	2.3-		
BGL_1c03700	hypothetical protein	2.5-		
BGL_1c03750	hypothetical protein	2.2-		
BGL_1c04130	hypothetical protein	2.7-		
BGL_1c04140	glutathione S-transferase	2.9-		
BGL_1c04160	aspartate aminotransferase AspB	4.4-		
BGL_1c04170	hypothetical protein	4.2-		
BGL_1c04180	transcriptional regulator, LysR family	3.6-		
BGL_1c04230	anthranilate synthase component 1 TrpE			2.5-
BGL_1c04720	ribosomal protein L27			2.5+
BGL_1c06060	putative flavohemo protein Hmp	3.2-		
BGL_1c06190	chaperone DnaK			2.1-
BGL_1c06220	3-methyl-2-oxobutanoate hydoxymethyltransferase PanB			2.2-
BGL_1c06430	capsular polysaccharide exportinner-membrane protein WcbD		2.2+	2.4+
BGL_1c06790	Protein of unknown function (DUF3318)			2.2-
BGL_1c06830	acyl-CoA dehydrogenase			2.1-
BGL_1c06860	4-hydoxybenzoate transporter PcaK	2.9+		
BGL_1c06890	cob(II)yrinic acid a,c-diamide reductase BluB	2.3-		
BGL_1c06900	transcriptional regulator, LysR family	14.0-		
BGL_1c07040	hypothetical protein	42.2-		4.9-
BGL_1c07150	rubredoxin RubA		2.0+	2.5+
BGL_1c07210	dihydroorotase-like protein PyrC			2.1-
BGL_1c07690	integrase		2.9+	2.8+
BGL_1c07900	transcriptional regulator, LysR family			13.0+
BGL_1c07910	N-acylhomoserine lactone synthase BpsI	2.9-		3.0+
BGL_1c07920	hypothetical protein			4.8+
BGL_1c07930	UspA domain protein			6.0+
BGL_1c07940	transcriptional regulator, LysR family			2.1+
BGL_1c07950	major facilitator superfamily MFS-1 transporter			2.4+
BGL_1c07980	autoinducer-binding transcriptional regulator, LuxR family protein	2.4-		
BGL_1c08000	thioesterase superfamily protein	2.1-		
BGL_1c08010	acyl-CoA dehydrogenase	2.5-		
BGL_1c08110	hypothetical protein	2.7-		2.4-
BGL_1c08430	hypothetical protein		29.8+	33.4+
BGL_1c08470	resolvase	2.1+		

Locus_tag	Predicted function	Fold change		
		BGPG2	BGPG3	BGPG4
BGL_1c08480	hypothetical protein		2.2+	2.2+
BGL_1c08690	porin Gram-negative type	2.3-		
BGL_1c08700	hypothetical protein	2.7-		
BGL_1c08710	cold-shock DNA-binding domain protein	10.1-	2.2+	2.5+
BGL_1c08720	DNA polymerase III, epsilon subunit	2.7-		
BGL_1c08750	transglycosylase-like protein	2.1-		
BGL_1c08830	xanthine dehydrogenase accessory protein XdhC			2.3-
BGL_1c08900	monooxygenase, FAD-binding			2.2-
BGL_1c08930	formate dehydrogenase, beta subunit			2.0-
BGL_1c08990	biodegradative arginine decarboxylase AdiA	8.9+		
BGL_1c09000	arginine/agmatine antiporter AdiC	23.5+		
BGL_1c09070	hypothetical protein		7.2+	9.9+
BGL_1c09860	ribosomal protein L32		2.1+	2.9+
BGL_1c11560	major facilitator superfamily MFS-1 transporter			2.5+
BGL_1c11780	para-aminobenzoate synthase PabB			2.1-
BGL_1c11970	HAD-superfamily hydrolase, subfamily IA, variant3	3.9+		
BGL_1c11980	major facilitator superfamily MFS-1 transporter			2.9+
BGL_1c12010	heat shock protein Hsp20		2.3-	3.4-
BGL_1c12020	heat shock protein Hsp20		2.7-	4.3-
BGL_1c12030	phosphoenolpyruvate carboxykinase	2.2+	2.1-	3.9-
BGL_1c12090	acetyl-CoA carboxylase beta subunit-like protein			2.7-
BGL_1c12100	malonate decarboxylase, gamma subunit MdcE			2.5-
BGL_1c12110	phosphoribosyl-dephospho-CoA transferase MdcG			3.0-
BGL_1c12630	MltA-interacting protein MipA family	2.2-		
BGL_1c12860	putative acyl-carrier-protein			2.1+
BGL_1c13180	hypothetical protein		3.2+	3.0+
BGL_1c13200	Rhs family protein	2.1-		
BGL_1c13390	type VI secretion system protein, Vgr family protein VgrG			2.1-
BGL_1c14040	sensor kinase protein RcsC	5.3+		
BGL_1c14340	transcriptional regulator, GntR family	2.2-		
BGL_1c14350	sodium:dicarboxylate symporter	2.4-		
BGL_1c14370	hypothetical protein	2.0-		
BGL_1c14410	hypothetical protein	2.2-	9.1+	2.5-
BGL_1c14460	hypothetical protein	4.0+		
BGL_1c14470	transcriptional regulatory protein CusR	3.6+		
BGL_1c14490	4-hydoxyphenylpyruvate dioxygenase	2.1+		
BGL_1c14710	putative mutator mutT protein	30.3-	2.7+	2.5+
BGL_1c14720	succinylglutamate desuccinylase/aspartoacylase	2.1-		
BGL_1c14740	beta-lactamase	2.1-		
BGL_1c14840	hypothetical protein		2.1+	
BGL_1c14870	Rhs element Vgr protein	2.2-		
BGL_1c14880	Rhs family protein	2.3-		

Locus_tag	Predicted function	Fold change		
		BGPG2	BGPG3	BGPG4
BGL_1c14890	hypothetical protein		2.0+	2.1+
BGL_1c14900	Immunity protein Imm1	2.0-		
BGL_1c15220	5-deoxy-glucuronate isomerase IolB	2.7+		
BGL_1c15230	putative amine catabolism-like protein	2.8+		
BGL_1c15240	3D-(3,5/4)-trihydoxycyclohexane-1,2-dione hydrolase IolD	3.1+		
BGL_1c15250	5-dehydro-2-deoxygluconokinase IolC	3.4+		
BGL_1c15260	sugar ABC transporter, ATP-binding protein	3.1+		
BGL_1c15270	sugar ABC transporter, permease protein	3.9+		
BGL_1c15280	sugar ABC transporter, periplasmic sugar-binding protein	5.7+	2.8+	
BGL_1c15290	SIS domain protein	2.9+	2.2+	2.4+
BGL_1c15300	inositol 2-dehydrogenase IdhA	2.2+		
BGL_1c15710	50S ribosomal protein L35			3.1+
BGL_1c15810	L-sulfolactate dehydrogenase ComC			2.0-
BGL_1c15950	hypothetical protein			3.3+
BGL_1c16020	hypothetical protein	2.1+		
BGL_1c16240	putative exported heme utilisation-like protein	6.9-		
BGL_1c16250	hypothetical protein	130.1-		3.0-
BGL_1c16260	hypothetical protein	274.1-		3.0-
BGL_1c16270	Flp/Fap pilin component	7.1-	2.4+	2.1+
BGL_1c16280	peptidase A24A, prepilin type IV	12.0-		
BGL_1c16290	TadE-like protein	11.1-		
BGL_1c16300	flp pilus assembly CpaB	10.2-		
BGL_1c16310	putative Flp pilus assembly protein secretin CpaC	9.5-		
BGL_1c16320	putative pilus assembly protein, CpaE-like protein	10.9-		
BGL_1c16330	putative Flp pilus assembly protein ATPase CpaF	12.4-		
BGL_1c16340	flp pilus assembly protein TadB	10.8-		
BGL_1c16350	Flp pilus assembly protein TadC	11.3-		
BGL_1c16360	flp pilus assembly protein TadD	10.5-		
BGL_1c16370	hypothetical protein	10.6-		
BGL_1c16380	sigma 54 specific transcriptional regulator, Fis family	7.4-		
BGL_1c16420	AMP-dependent synthase and ligase			4.0-
BGL_1c17000	death-on-curing family protein	3.0+	2.9+	2.9+
BGL_1c17290	hypothetical protein	2.6-		
BGL_1c17300	nucleoside 2-deoxyribosyltransferase	2.1-		
BGL_1c17730	hypothetical protein	2.3+		
BGL_1c17810	putative bacteriophage protein	2.6-	6.8-	6.6-
BGL_1c17820	hypothetical protein	3.2-	8.4-	8.3-
BGL_1c17830	putative bacteriophage protein	2.0-	3.8-	3.3-
BGL_1c17840	putative bacteriophage protein	2.8-	4.4-	4.9-
BGL_1c17860	phage-like tail protein		2.0-	2.2-
BGL_1c17870	hypothetical protein		2.1-	2.6-
BGL_1c17890	hypothetical protein		2.0-	2.5-

Locus_tag	Predicted function	Fold change		
		BGPG2	BGPG3	BGPG4
BGL_1c17900	putative bacteriophage protein		3.0-	3.1-
BGL_1c17920	putative phage baseplate assembly protein		2.3-	2.9-
BGL_1c17940	hypothetical protein		2.0-	
BGL_1c17990	lysozym	9.0-	9.0-	5.3-
BGL_1c18200	ABC-type sugar transport system, ATPase component		2.2-	2.8-
BGL_1c18220	transcriptional regulator, LacI family			2.0-
BGL_1c18230	ribokinase			2.2-
BGL_1c18240	protein kinase			2.8-
BGL_1c18600	methyl-accepting chemotaxis protein	4.0-		
BGL_1c18610	hypothetical protein		3.3+	2.5+
BGL_1c18690	hypothetical protein		7.7+	8.4+
BGL_1c18740	hypothetical protein			2.3+
BGL_1c18770	hypothetical protein	2.4-		
BGL_1c18780	hypothetical protein	2.4-		
BGL_1c18810	CRISPR-associated protein Cas1	2.2-		
BGL_1c18820	CRISPR-associated helicase Cas3, Yersinia-type	3.1-		
BGL_1c18830	CRISPR-associated protein, Csy1 family	8.3-	3.3-	4.2-
BGL_1c18840	CRISPR-associated protein, Csy2 family	9.5-	3.9-	5.5-
BGL_1c18850	CRISPR-associated protein, Csy3 family	7.5-	3.1-	3.7-
BGL_1c18860	CRISPR-associated protein, Csy4 family	6.7-	2.7-	3.1-
BGL_1c18870	hypothetical protein	3.2-		
BGL_1c19000	non-ribosomal peptide synthase	2.4-		
BGL_1c19170	hypothetical protein		2.8+	3.0+
BGL_1c19330	hypothetical protein	2.4-		
BGL_1c19370	phosphocarrier HPr protein	2.6+		
BGL_1c19520	major facilitator superfamily MFS-1 transporter	2.4+		
BGL_1c19540	putative gluconolactonase			2.2-
BGL_1c19770	hypothetical protein	175.0-		
BGL_1c19850	lactate utilization protein A		2.5-	2.2-
BGL_1c19900	hypothetical protein			2.5-
BGL_1c19910	short chain dehydrogenase			2.2-
BGL_1c19920	mandelate racemase	2.0+		
BGL_1c19940	peptidase, M1 family protein	6.3-		
BGL_1c20290	hypothetical protein	2.7-		
BGL_1c20300	putative esterase	3.9-		
BGL_1c20310	beta-ketoacyl synthase	2.6-		
BGL_1c20330	hypothetical protein	2.7-		
BGL_1c20340	major facilitator superfamily MFS-1 transporter	2.6-		
BGL_1c20350	4Fe-4S ferredoxin, iron-sulfur binding domain protein FdxA	2.8-		
BGL_1c20360	hypothetical protein	2.8-		
BGL_1c20370	putative GCN5-like N-acetyltransferase	3.3-		
BGL_1c20380	hypothetical protein	3.1-		

Locus_tag	Predicted function	Fold change		
		BGPG2	BGPG3	BGPG4
BGL_1c20390	AMP-dependent synthase and ligase	3.3-		
BGL_1c20400	hypothetical protein	2.8-		
BGL_1c20530	TonB-dependent Fe(III)-pyochelinreceptor	2.0-		
BGL_1c20760	serine metalloprotease	17.5-		
BGL_1c20770	Fungal chitosanase of glycosyl hydrolase group 75	2.1-		
BGL_1c20950	metallophosphoesterase			2.4-
BGL_1c20970	hypothetical protein		2.1+	2.1+
BGL_1c21030	YbaK/prolyl-tRNA synthase associated region	2.2-		
BGL_1c21040	linear gramicidin synthase subunit C	2.9-		
BGL_1c21050	linear gramicidin dehydrogenase LgrE	2.4-		
BGL_1c21060	hypothetical protein, acyl carrier protein-like, phophopantheine binding domain	2.5-		
BGL_1c21070	protein CmaB	5.3-		
BGL_1c21090	putative periplasmic substrate-binding protein			2.1-
BGL_1c21170	hypothetical protein	3.2+		
BGL_1c21180	hypothetical protein	3.3+		
BGL_1c21240	hypothetical protein	5.5-		
BGL_1c21470	2-dehydro-3-deoxygluconokinase KdgK			2.1-
BGL_1c21750	cystathionine beta-lyase MetC			2.5-
BGL_1c22090	phosphatidylserine decarboxylase-like protein	2.5-		
BGL_1c22110	hypothetical protein	17.6-		
BGL_1c22350	small nuclear ribonucleo protein (Sm protein)		2.1+	2.3+
BGL_1c22680	acetoin catabolism regulatory protein AcoR			2.3-
BGL_1c22710	ATP-dependent Clp protease, ATP-binding subunit ClpB			2.2-
BGL_1c23260	hypothetical protein			2.2-
BGL_1c23490	putative class-V aminotransferase			2.3-
BGL_1c23590	transport-associated protein	3.0-		
BGL_1c23640	ABC sugar transporter, ATPase protein			2.1-
BGL_1c23700	sensor histidine kinase			2.6-
BGL_1c23840	alkyl hydroperoxide reductase AhpC			2.1-
BGL_1c23870	acetylornithine deacetylase ArgE			2.0-
BGL_1c24010	ureidoglycolate hydrolase			2.5-
BGL_1c24090	CMP/dCMP deaminase, zinc-binding	12.5+		
BGL_1c24100	hypothetical protein	9.5+		
BGL_1c24220	hypothetical protein		5.0+	3.7+
BGL_1c24870	acyl-CoA-binding protein		2.2+	2.0+
BGL_1c24880	DEAD/DEAH box helicase-like protein	3.3+		2.2+
BGL_1c24890	isocitrate lyase AceA			2.0-
BGL_1c25050	anaerobic dehydrogenase			2.5-
BGL_1c25200	hypothetical protein	6.4-		
BGL_1c25310	ABC-type polar amino acid transport system periplasmic substrate-binding protein			2.6+

Locus_tag	Predicted function	Fold change		
		BGPG2	BGPG3	BGPG4
BGL_1c26330	phasin-like protein	2.5-		2.1+
BGL_1c26400	oligopeptidase A			2.1-
BGL_1c26420	putative transmembrane efflux protein	4.7-		
BGL_1c26430	argininosuccinate synthase ArgG	10.2-		
BGL_1c26440	putative non-ribosomal peptide synthase, acetyl-CoA ligase-like protein	8.1-		
BGL_1c26450	aldo/keto reductase	7.0-		
BGL_1c26460	adenylylsulfate kinase	5.7-		
BGL_1c26500	oxidoreductase, molybdopterin binding		7.6+	3.1-
BGL_1c26510	putative transmembrane hydrogenase cytochrome b-type subunit		18.0+	3.0-
BGL_1c26710	putative lipoprotein			2.1-
BGL_1c26720	aminotransferase, class III	3.2-		
BGL_1c26730	gamma-glutamylputrescine synthase PuuA	2.7-		
BGL_1c26740	gamma-glutamyl-gamma-aminobutyrate hydrolase PuuD	3.0-		
BGL_1c26750	hypothetical protein	2.0-		
BGL_1c26770	gamma-glutamyl-gamma-aminobutyraldehyde dehydrogenase PuuC			2.0-
BGL_1c26780	protein HutG	2.1+		
BGL_1c26790	formimidoylglutamate deiminase HutF	2.7+		
BGL_1c26810	putative acyl-CoA dehydrogenase HutD		2.0-	
BGL_1c26830	histidine utilization repressor HutC	3.6+		
BGL_1c26840	histidine ammonia-lyase HutH	3.1+		
BGL_1c26970	putative plasmid recombination enzyme		10.5+	8.5+
BGL_1c27180	bacterioferritin-associated ferredoxin			2.3+
BGL_1c27300	hypothetical protein			2.9+
BGL_1c27570	acyl-CoA dehydrogenase			2.2-
BGL_1c27800	30S ribosomal protein S15			2.6+
BGL_1c27820	carbonate dehydratase	6.9-		
BGL_1c27830	sulphate transporter	9.5-		
BGL_1c27880	hypothetical protein		2.7+	4.7+
BGL_1c28240	hypothetical protein	2.0-		
BGL_1c28280	hypothetical protein	2.0-		2.0-
BGL_1c28530	hypothetical protein	2.1-	5.7+	5.7+
BGL_1c28560	cytochrome c family protein	2.3-		
BGL_1c28570	cytochrome c553	2.3-		
BGL_1c28660	G/U mismatch-specific uracil-DNA glycosylase (EC 3.2.2.-)			2.3-
BGL_1c28670	chorismate lyase (EC 4.1.3.40)			2.3-
BGL_1c28680	molecular chaperone, HSP90 family protein			2.2-
BGL_1c28810	Domain of unknown function (DUF4399)	2.2-		
BGL_1c28970	hypothetical protein		2.5+	2.5+
BGL_1c28980	hypothetical protein	2.1-		

Locus_tag	Predicted function	Fold change		
		BGPG2	BGPG3	BGPG4
BGL_1c28990	hypothetical protein		2.2+	2.1+
BGL_1c29070	hypothetical protein			2.5-
BGL_1c29080	hypothetical protein			2.1+
BGL_1c29170	hypothetical protein			3.1-
BGL_1c29180	alpha,alpha-trehalose-phosphate synthase OtsA			2.7-
BGL_1c29250	hypothetical protein		2.9+	3.0+
BGL_1c29560	surface antigen (D15)	2.0-		
BGL_1c29970	hypothetical protein		2.2+	2.3+
BGL_1c30010	acetyltransferase, GNAT family			2.0-
BGL_1c30020	ABC transporter-like protein	3.1+		
BGL_1c30030	branched-chain amino acid ABC transporter, ATP-binding protein	3.1+		
BGL_1c30040	inner-membrane translocator	3.2+		
BGL_1c30050	ABC branched-chain amino acid family transporter, inner membrane protein	3.2+		
BGL_1c30100	50S ribosomal protein L33		3.1+	5.0+
BGL_1c30260	multicopper oxidase family protein			2.2-
BGL_1c30580	30S ribosomal protein S20		2.4+	3.9+
BGL_1c30670	methylated-DNA--protein-cysteinemethyl transferase Ogt			2.3-
BGL_1c30730	thioredoxin			2.1-
BGL_1c30750	pyridoxine/pyridoxamine 5'-phosphate oxidase PdxH	2.2+		
BGL_1c31020	zinc-containing alcohol dehydrogenasesuperfamilyprotein		2.2+	2.1+
BGL_1c31140	sterol desaturase family protein		2.4+	
BGL_1c31150	phospholipase/lecithinase/hemolysin-likeprotein	2.1+		
BGL_1c31330	hypothetical protein	2.3-		2.4-
BGL_1c31340	hypothetical protein	2.5-		2.6-
BGL_1c31350	Rhs element Vgr protein	2.7-		
BGL_1c31500	transcriptional regulator, ArgP family	18.1-		
BGL_1c31660	dTDP-glucose 4,6-dehydratase RfbB	5.5-	2.5+	
BGL_1c31940	putative cytochrome c4	2.2-		
BGL_1c31950	putative periplasmic cytochrome ccontainingprotein	2.7-		
BGL_1c32020	hypothetical protein	3.4-		
BGL_1c32050	glycerol kinase GlpK			2.0-
BGL_1c32150	tetratricopeptide TPR_2 repeat protein			2.0-
BGL_1c32640	hypothetical protein	5.3+		
BGL_1c33180	cytochrome bd ubiquinol oxidase, subunit I		2.4-	3.2-
BGL_1c33190	cytochrome d ubiquinol oxidase subunit 2		2.4-	3.4-
BGL_1c33400	hypothetical protein	2.4-		
BGL_1c33410	cytochrome c oxidase subunit 3 CtaE	2.5-		
BGL_1c33420	Protein of unknown function (DUF2970)	2.2-		
BGL_1c33430	cytochrome c oxidase assembly protein CtaG	2.4-		
BGL_1c33720	C4-dicarboxylate transport protein DctA	2.6+		

Locus_tag	Predicted function	Fold change		
		BGPG2	BGPG3	BGPG4
BGL_1c34000	hypothetical protein		2.5+	3.2+
BGL_1c34010	phospholipase, patatin family protein			2.0-
BGL_1c34340	beta and gamma crystallin	5.3-		
BGL_1c34500	N-acylglucosamine 2-epimerase family protein		2.7-	3.2-
BGL_1c34970	flagellar basal-body rod protein FlgG		2.2+	3.1+
BGL_1c34990	flagellar hook protein FlgE		2.4+	4.1+
BGL_1c35000	flagellar hook capping protein FlgD		2.4+	3.6+
BGL_1c35020	flagellar basal-body rod protein FlgB		2.4+	4.4+
BGL_1c35300	PepSY-associated TM helix family protein			2.2+
BGL_1c35340	flagellar protein FliS		2.1+	2.0+
BGL_1c35360	flagellar M-ring protein FliF		3.6+	5.3+
BGL_1c35370	flagellar motor switch protein FliG		2.1+	2.7+
BGL_1c35380	flagellar assembly protein FliH		2.5+	3.6+
BGL_1c35400	flagellar export protein FliJ		3.2+	3.8+
BGL_1c35440	NAD-dependent aldehyde dehydrogenase			2.6-
BGL_1c35870	queuosine biosynthesis protein QueD	2.0+		
BGL_1c36080	hypothetical protein	4.4-		2.2+
BGL_1c36090	hypothetical protein	5.5-	2.2+	3.1+
BGL_1c36100	major facilitator superfamily MFS-1 transporter	3.3-		
BGL_2c00060	hypothetical protein	3.0-		
BGL_2c00170	major facilitator superfamily MFS-1 transporter			2.4-
BGL_2c00180	glucarate dehydratase	2.1+		
BGL_2c00190	5-dehydro-4-deoxyglucarate dehydratase	2.6+		
BGL_2c00250	hypothetical protein	2.2-		
BGL_2c00970	putative antibiotic synthase, amino acid adenylation domain	2.3-		
BGL_2c01450	dimethyl sulfoxide reductase DmsA			2.7-
BGL_2c01470	putative alcohol dehydrogenase, cytochrome c subunit			2.5-
BGL_2c01540	D-mannonate oxidoreductase UxuB	2.4+		
BGL_2c01560	starvation-sensing protein RspA			2.3-
BGL_2c01820	hypothetical protein		2.1+	
BGL_2c01940	hypothetical protein		3.7+	5.0+
BGL_2c01960	putative periplasmic substrate-binding protein	4.6-		
BGL_2c01980	putative monooxygenase MoxC	3.4-		
BGL_2c02100	'putative HpaB; type III secretion chaperone, CesT family'		3.4+	3.7+
BGL_2c02450	tyrocidine synthase 2	2.1-	2.3+	3.5+
BGL_2c02460	phosphopantetheine attachment site domain protein, BarA-like		2.6+	3.9+
BGL_2c02470	chlorinating enzyme, SyrB2/BarB2-like protein	2.9-		2.1+
BGL_2c02480	thioesterase type II, NRPS/PKS/S-FAS family, BarC-like	2.7-		
BGL_2c02490	putative transferase	2.2-		
BGL_2c02500	branched-chain amino acid aminotransferase IlvE	2.7-		2.0+

Locus_tag	Predicted function	Fold change		
		BGPG2	BGPG3	BGPG4
BGL_2c02510	thioesterase type II, NRPS/PKS/S-FAS family, BarC-like	2.2-		
BGL_2c02520	acetaldehyde dehydrogenase (acetylating)	2.2-	2.0+	
BGL_2c02530	4-hydoxy-2-oxovalerate aldolase family	2.4-		2.1+
BGL_2c02540	hypothetical protein, RmlC-like cupins superfamily			2.2+
BGL_2c02550	putative translocator protein, LysE family	2.2-		
BGL_2c02560	putative flavin reductase domain-containing protein	2.2-		
BGL_2c03190	putative alcohol dehydrogenase, zinc-binding domain protein			2.1-
BGL_2c03510	putative lipoprotein		2.2+	2.0+
BGL_2c03650	putative L-sorbosone dehydrogenase	6.5-	3.6-	6.2-
BGL_2c03660	hypothetical protein	7.0-	3.1-	5.2-
BGL_2c03670	putative transcriptional regulator, LysR family	2.2-	2.2-	
BGL_2c03680	isoquinoline 1-oxidoreductase subunit alpha	2.4-	2.4-	3.1-
BGL_2c03690	isoquinoline 1-oxidoreductase subunit beta		3.3-	3.7-
BGL_2c03700	putative membrane-bound alcohol dehydrogenase, cytochrome c subunit		3.1-	4.0-
BGL_2c03710	putative XdhC-CoxI family protein		3.7-	4.6-
BGL_2c03720	putative transcriptional regulator, LysR family	2.3-		2.3-
BGL_2c03780	hypothetical protein			2.4-
BGL_2c03790	putative transcriptional regulator, LysR family	2.3-		
BGL_2c03800	putative aldo/keto reductase	3.0-	3.1-	2.9-
BGL_2c03810	branched-chain amino acid aminotransferase	2.0-	2.1-	2.3-
BGL_2c03830	Mn2+/Fe2+ transporter NRAMP family	22.4-	16.6-	12.4-
BGL_2c03840	hypothetical protein	91.8-	55.8-	104.1-
BGL_2c03850	cysteine synthase CysB	69.9-	54.3-	51.5-
BGL_2c03860	putative SAM-dependent methyltransferase	128.0-	53.3-	67.4-
BGL_2c03870	carbamoyltransferase family protein	111.4-	63.7-	63.7-
BGL_2c03880	putative alpha/beta hydrolase fold protein	131.0-	60.5-	98.3-
BGL_2c03890	putative luciferase family protein	108.4-	39.2-	42.7-
BGL_2c03900	hypothetical protein	225.7-	148.4-	193.4-
BGL_2c03910	putative phytanoyl-CoA dioxygenase family protein	149.8-	50.9-	52.9-
BGL_2c03920	ABC transporter family protein, ATP-binding and transmembrane domain	32.5-	18.8-	22.5-
BGL_2c03930	hypothetical protein	23.8-	18.9-	26.8-
BGL_2c03940	putative non-ribosomal peptide synthase	22.8-	18.8-	25.5-
BGL_2c03950	putative aminoglycoside phosphotransferase	33.6-	21.0-	23.2-
BGL_2c03960	putative thiopurine S-methyltransferase family protein	24.8-	8.4-	17.5-
BGL_2c03970	hypothetical protein	15.7-	4.5+	5.7-
BGL_2c03980	acyl-homoserine-lactone synthase LasI	10.5-	68.3-	5.7-
BGL_2c03990	transcriptional activator protein LasR	5.9-		4.3-
BGL_2c04000	ABC transporter, ATP-binding/permease protein			2.7-
BGL_2c04010	hypothetical protein			2.5-

Locus_tag	Predicted function	Fold change		
		BGPG2	BGPG3	BGPG4
BGL_2c04020	putative 4Fe-4S ferredoxin, iron-sulfur binding domain protein		2.1-	2.9-
BGL_2c04050	transcriptional regulator, IclR family	2.0-	2.0-	2.1-
BGL_2c04070	methyl-accepting chemotaxis sensory transducer with Pas/Pac sensor	2.1-		
BGL_2c04090	succinate-semialdehyde dehydrogenase (NADP+)	2.4-		
BGL_2c04100	4-aminobutyrate aminotransferase	2.9-		
BGL_2c04110	transcriptional regulator, GntR family		2.3-	2.8-
BGL_2c04130	hypothetical protein	2.1-		2.0-
BGL_2c04190	hypothetical protein	2.7-		
BGL_2c04200	transposase IS3/IS911 family protein	2.6-		
BGL_2c04210	putative transposase	2.9-	2.1-	2.5-
BGL_2c04220	putative transposase	2.5-		
BGL_2c04230	putative type VI secretion system Vgr family protein	2.6-	2.5-	3.7-
BGL_2c04250	putative membrane protein	9.1-	2.8-	2.3-
BGL_2c04260	selenide, water dikinase SelD	2.6-	2.5-	2.8-
BGL_2c04280	cation diffusion facilitator family transporter		2.3-	2.4-
BGL_2c04290	putative purine nucleoside permease	4.3-	2.7-	4.1-
BGL_2c04300	putative exported protein	6.9-	3.3-	2.6-
BGL_2c04310	aspartate-proton symporter YveA	2.0-	2.0-	2.2-
BGL_2c04320	putative malate/L-lactate dehydrogenase	2.3-	2.4-	2.6-
BGL_2c04330	transcriptional regulator, LysR family		2.5-	2.9-
BGL_2c04340	alpha/beta hydrolase	2.6-	2.6-	3.3-
BGL_2c04350	RND efflux transporter, MFP subunit	3.8-	4.8-	3.6-
BGL_2c04360	RND efflux transporter, hydrophobe/amphiphile efflux-1 (HAE1) family	3.5-	3.6-	3.8-
BGL_2c04370	RND efflux transporter, outer membrane factor (OMF) lipoprotein, NodT family	2.1-	3.0-	3.3-
BGL_2c04400	hypothetical protein	2.0-	2.4-	2.2-
BGL_2c04410	acyl-CoA dehydrogenase	2.6-	2.6-	2.6-
BGL_2c04420	hypothetical protein	3.4-		
BGL_2c04450	transcriptional regulatory protein QseB	2.1-	2.7-	3.2-
BGL_2c04460	sensor protein QseC		2.2-	2.5-
BGL_2c04470	phosphoserine transaminase	2.2-	2.5-	3.2-
BGL_2c04480	two component heavy metal response transcriptional regulator, winged helix family		2.2-	2.8-
BGL_2c04490	heavy metal sensor signal transduction histidine kinase		2.6-	3.1-
BGL_2c04610	D-galactonate transporter, MFS transport family	3.5-	8.2-	7.3-
BGL_2c04620	aldehyde dehydrogenase	5.0-	8.8-	7.7-
BGL_2c04630	tryptophanyl-tRNA synthase TrpS		3.8-	4.9-
BGL_2c04640	5-dehydro-4-deoxyglucarate dehydratase	3.7-	4.3-	2.8-
BGL_2c04650	D-galactarate dehydratase GarD		5.6-	5.2-

Locus_tag	Predicted function	Fold change		
		BGPG2	BGPG3	BGPG4
BGL_2c04660	hypothetical protein	3.3-		3.1-
BGL_2c04670	acetyltransferase	3.8-		
BGL_2c04680	hypothetical protein, DoxX family	6.2-		
BGL_2c04710	proline racemase		2.0-	3.2-
BGL_2c04750	transcriptional regulator, AraC family	3.0-	2.1-	2.3-
BGL_2c04770	4-hydoxyphenylpyruvate dioxygenase	4.0-	6.0-	4.9-
BGL_2c04780	3-dehydroquinate dehydratase AroQ, type-II 3-dehydroquinase family		2.1-	2.3-
BGL_2c04790	shikimate 5-dehydrogenase AroE		2.6-	3.1-
BGL_2c04800	putative glucarate transporter GudP		2.1-	2.6-
BGL_2c04810	2-aminoethylphosphonate-pyruvate transaminase PhnW	8.4-	6.2-	14.3-
BGL_2c04820	2-aminoethylphosphonate ABC transport, periplasmic substrate-binding protein PhnS	4.2-	4.6-	5.2-
BGL_2c04830	2-aminoethylphosphonate ABC transport, ATP-binding protein PhnT	4.7-	2.1-	2.2-
BGL_2c04840	2-aminoethylphosphonate ABC transport, permease protein PhnU	3.9-		2.1-
BGL_2c04850	2-aminoethylphosphonate ABC transport, membrane protein PhnV	3.2-		2.4-
BGL_2c04860	phosphonoacetate hydrolase PhnA	4.1-	3.6-	6.0-
BGL_2c04870	HD phosphohydrolase-like protein	4.5-	3.0-	4.8-
BGL_2c04930	hypothetical protein		2.5+	6.0+
BGL_2c05050	serine-type carboxypeptidase			2.1-
BGL_2c05630	long-chain-fatty-acid--CoA ligase FadD	2.2-	2.2+	3.3+
BGL_2c05670	putative membrane-anchored cell surface protein	36.9-		2.5-
BGL_2c05770	hydoxylamine reductase , hybrid-cluster protein	2.8-		
BGL_2c05800	putative hydoxypyruvate isomerase			2.3-
BGL_2c05930	flavin-type monooxygenase			2.3-
BGL_2c06160	tyrosine-specific transport protein TyrP	2.5+		
BGL_2c06180	glutamate/gamma-aminobutyrate antiporter	3.0+	2.7-	
BGL_2c06390	L-serine dehydratase SdaA		2.4+	
BGL_2c06420	sarcosine oxidase subunit alpha SoxA	2.0+	2.4+	
BGL_2c06440	dihydroneopterin aldolase		2.0+	2.2-
BGL_2c06460	CsbD family protein		3.7+	3.4+
BGL_2c06600	NADH:flavin oxidoreductase/NADH oxidase			2.3-
BGL_2c06700	phosphoesterase family protein	2.2-		
BGL_2c07030	alpha/beta hydrolase	2.1-		
BGL_2c07040	hypothetical protein	2.2-		
BGL_2c07100	peptidase S1 and S6, chymotrypsin/Hap			2.0-
BGL_2c07470	rhamnosyltransferase I subunit A	26.0-		
BGL_2c07480	rhamnosyltransferase I subunit B	30.7-		
BGL_2c07490	multidrug resistance protein B	18.9-		

Locus_tag	Predicted function	Fold change		
		BGPG2	BGPG3	BGPG4
BGL_2c07500	rhamnosyltransferase I subunit C	29.9-		
BGL_2c07510	RND efflux transporter, outer membrane factor (OMF) lipoprotein, NodT family	16.3-		
BGL_2c07520	secretion protein, HlyD family	4.0-		
BGL_2c07530	chitinase	12.5-		
BGL_2c07540	aldolase, isopropylmalate synthase-like protein	69.6-		
BGL_2c07550	hypothetical protein, haem oxygenase-like	16.4-		2.0+
BGL_2c07560	branched-chain amino acid aminotransferase IlvE	14.1-		
BGL_2c07570	hypothetical protein, N-acetylglucosaminylphosphatidylinositol de-N-acetylase-like	9.2-		
BGL_2c07580	phenazine biosynthesis-like protein	10.9-		
BGL_2c07590	phosphoribosylglycinamide synthase	5.9-		
BGL_2c07600	tetracycline resistance protein TetA	3.9-		
BGL_2c07900	hypothetical protein		2.9+	3.2+
BGL_2c07940	hypothetical protein			2.1-
BGL_2c08030	3-hydoxybutyrate dehydrogenase BdhA			3.2-
BGL_2c08040	enoyl-CoA hydratase			3.1-
BGL_2c08060	DNA binding protein	4.3-		
BGL_2c08120	glycoside hydrolase-like protein	10.7-		
BGL_2c08160	'penicillin-binding protein, 1A family; PbP-1a; PBP1a'		2.0-	2.7-
BGL_2c08180	putative chitinase	2.5-		
BGL_2c08450	hypothetical protein		2.4+	2.5+
BGL_2c08490	transcriptional regulator, LacI family	3.8-		
BGL_2c08880	hypothetical protein	2.4+		
BGL_2c08910	threonine ammonia-lyase, biosynthetic	2.4+		2.4+
BGL_2c08980	hypothetical protein		2.5+	2.2+
BGL_2c09000	PepSY amd peptidase M4 protein	2.4-		
BGL_2c09010	TonB-dependent siderophore receptor	3.3-		
BGL_2c09060	hypothetical protein		2.1+	
BGL_2c09510	putative exported protein		2.7+	3.3+
BGL_2c09540	methyl-accepting chemotaxis sensory transducer	2.2-		
BGL_2c09820	allophanate hydrolase	3.0+		
BGL_2c09830	transcriptional regulator, LysR family	2.5+		
BGL_2c09850	N-hexanoyl homoserine lactone synthase RhlL/TofI	4.1-		
BGL_2c09860	hypothetical protein	25.1-		
BGL_2c09870	N-octanoyl homoserine lactone synthase RhlR/TofR	5.6+		
BGL_2c09990	amidase		2.0-	2.6-
BGL_2c10110	hydrolase, epoxide hydrolase-like		2.0-	
BGL_2c10120	metal-dependent hydrolase	2.0+		
BGL_2c10130	short chain dehydrogenase	2.4+		
BGL_2c10140	flavin-containing monooxygenase	2.5+		

Locus_tag	Predicted function	Fold change		
		BGPG2	BGPG3	BGPG4
BGL_2c10150	transcriptional regulator, LysR family	2.3-		
BGL_2c10160	hypothetical protein	2.4-		
BGL_2c10170	hypothetical protein	4.0+		
BGL_2c10520	2-dehydropantoate 2-reductase PanE			2.0-
BGL_2c10540	putative cyanate transport protein cynX, MFS family	2.1-		
BGL_2c10630	hypothetical protein			2.3-
BGL_2c10640	hypothetical protein		2.1-	3.6-
BGL_2c10650	hypothetical protein			2.6-
BGL_2c10660	hypothetical protein			2.5-
BGL_2c10690	transcriptional regulator, LysR family			2.2-
BGL_2c10910	oxalyl-CoA decarboxylase Oxc			2.9-
BGL_2c10920	formyl-CoA:oxalate CoA-transferase Frc			2.9-
BGL_2c10940	hypothetical protein			2.8-
BGL_2c10950	hypothetical protein			2.7-
BGL_2c11060	alpha-L-arabinofuranosidase B	4.5-		
BGL_2c11280	TRAP dicarboxylate transporter, DctP subunit	2.0+		
BGL_2c11580	hypothetical protein, transglutaminase-like			2.1-
BGL_2c11690	channel protein, hemolysin III family protein	2.2+		
BGL_2c11830	putative periplasmic substrate-binding protein	2.6+		
BGL_2c11950	Thermostable hemolysin	2.3-	2.3+	3.3+
BGL_2c11990	flavin-dependent oxidoreductase-like protein			2.8+
BGL_2c12190	quinoprotein glucose dehydrogenase Gcd	4.5-		
BGL_2c12340	putative exported protein	3.1+		
BGL_2c12350	alpha/beta hydrolase	3.0+		
BGL_2c12360	putative glyoxalase (dioxygenase domain)	2.9+		
BGL_2c12370	uncharacterized Rieske-protein, [2Fe-2S] centre	2.4+		
BGL_2c12380	monooxygenase FAD-binding, aromatic-ring hydoxylase-like	2.6+		
BGL_2c12390	MFS general substrate transporter	2.1+		
BGL_2c12400	putative cyclase	2.0+		
BGL_2c12420	putative fumarylacetoacetate hydrolase			2.2-
BGL_2c12620	putative mandelate racemase	2.3+		
BGL_2c12640	D-galactonate transporter, MFS transport family	2.2+		
BGL_2c12870	thiamine pyrophosphate enzyme-like TPP bindingregion			2.2-
BGL_2c12970	putative hippurate hydrolase		2.3-	3.9-
BGL_2c13840	hypothetical protein			2.0-
BGL_2c13850	hypothetical protein			2.5-
BGL_2c13860	hypothetical protein			2.3-
BGL_2c13870	30S ribosomal protein S21			2.1-
BGL_2c13890	cold shock-like protein CspA	2.7+	5.7+	3.7+
BGL_2c13950	putative signal peptide protein	2.1-		
BGL_2c13960	tetratricopeptide TPR_2	2.4-		

Locus_tag	Predicted function	Fold change		
		BGPG2	BGPG3	BGPG4
BGL_2c13970	putative lipoprotein	2.7-		
BGL_2c13980	putative transmembrane protein	2.5-		
BGL_2c13990	hypothetical transmembrane protein	2.3-		
BGL_2c14000	glycosyl transferase, group 1	2.6-		
BGL_2c14010	putative transmembrane protein	2.7-		
BGL_2c14020	hypothetical protein	2.3-		
BGL_2c14110	hypothetical protein		2.4+	2.1+
BGL_2c14120	putative transcriptional regulator, GntR familyprotein	2.6-		2.0+
BGL_2c14140	alkylhydroperoxidase AhpD-like protein	3.0-		
BGL_2c14150	acyl-CoA synthases (AMP-forming)/AMP-acid ligase II-like protein	2.7-		
BGL_2c14160	phenylacetate-CoA oxygenase, PaaA subunit	3.2-		
BGL_2c14250	anthranilate synthase component 1 TrpE	2.4-		
BGL_2c14530	hypothetical protein	2.1+		
BGL_2c14540	putative lipoprotein	2.1+		
BGL_2c14550	putative lipoprotein			2.1-
BGL_2c14690	hypothetical protein		2.8+	2.6+
BGL_2c14880	hypothetical protein	11.8-		
BGL_2c14890	hypothetical protein	6.6-		
BGL_2c14900	TonB-dependent siderophore receptor			4.0+
BGL_2c15120	methyl-accepting chemotaxis sensory transducer	3.6-		
BGL_2c15150	glyoxalase/bleomycin resistanceprotein/dioxygenase			2.3-
BGL_2c15220	hypothetical protein		6.0+	6.1+
BGL_2c15240	NADH oxidoreductase containing a GroES-like domain			2.3-
BGL_2c15420	Protein of unknown function (DUF3304)		2.8+	2.3+
BGL_2c15550	hypothetical protein	5.3-		
BGL_2c15730	ABC transporter-like protein	2.9+		
BGL_2c15830	hypothetical protein	3.2-		
BGL_2c15840	hypothetical protein	4.0-		
BGL_2c15850	mandelate racemase	3.9-		
BGL_2c16310	GCN5-like N-acetyltransferase			2.3-
BGL_2c16320	D-isomer specific 2-hydoxyacid dehydrogenase, NAD-binding			2.0-
BGL_2c16410	drug resistance transporter, EmrB/QacA subfamily	305.1-		
BGL_2c16420	secretion protein, HlyD family	368.3-		
BGL_2c16430	RND efflux system, outer membrane lipoprotein, NodT family	313.9-		
BGL_2c16450	polyketide cyclase/dehydrase	74.8-		
BGL_2c16460	putative non-ribosomal peptide synthase	98.5-		
BGL_2c16470	hypothetical protein	214.4-		
BGL_2c16480	hypothetical protein, RmlC-like cupins superfamily	327.6-	2.2-	
BGL_2c16490	hypothetical protein	397.5-		

Locus_tag	Predicted function	Fold change		
		BGPG2	BGPG3	BGPG4
BGL_2c16500	transcriptional regulator, AraC family	19.8-		
BGL_2c16510	transcriptional regulator, LuxR family	4.1-		2.1+
BGL_2c16800	nitrite reductase [NAD(P)H] small subunit	2.3-		2.8-
BGL_2c16810	nitrite reductase [NAD(P)H] large subunit	2.1-	2.5-	3.1-
BGL_2c17020	hypothetical protein	2.1-		
BGL_2c17290	hypothetical protein			2.1-
BGL_2c17410	hypothetical protein		6.1+	
BGL_2c17420	hypothetical protein		6.5+	2.0-
BGL_2c17470	drug resistance transporter, EmrB/QacA family	5.1-	2.0+	
BGL_2c17500	transcriptional regulator, LysR family	2.0-		
BGL_2c17680	glutamate-1-semialdehyde 2,1-aminomutase HemL	2.3-		
BGL_2c17690	hypothetical protein	6.7-		
BGL_2c17700	putative non-ribosomal peptide synthase	2.9-		
BGL_2c18200	succinate dehydrogenase cytochrome b556 subunit		2.1+	2.5+
BGL_2c18330	FAD/FMN-containing dehydrogenases	2.1+		
BGL_2c18550	putative mutt/nudix hydrolase	2.2-		
BGL_2c18660	lipase LipA	2.5-		2.3-
BGL_2c18690	hypothetical protein		2.3+	2.6+
BGL_2c18700	hypothetical protein	2.1-		
BGL_2c18890	transcriptional regulator, GntR family	2.1-		
BGL_2c18910	PPE-repeat protein	4.7-		
BGL_2c18920	hypothetical protein	2.4-		
BGL_2c18940	hypothetical protein	6.0-		
BGL_2c18980	pseudomonalisin	10.9-		
BGL_2c19200	acyltransferase		2.4+	2.5+
BGL_2c19430	methyl-accepting chemotaxis sensory transducer	5.2-		
BGL_2c19440	hypothetical protein	5.3-		
BGL_2c20210	hypothetical protein	2.9-		
BGL_2c20220	putative phospholipase D / transphosphatidylase	9.1-		
BGL_2c20230	hypothetical protein	108.5-		
BGL_2c20240	hypothetical protein	28.5-		
BGL_2c20250	hypothetical protein	27.7-	2.0+	
BGL_2c20260	RNA polymerase sigma factor 70	24.0-	2.0+	
BGL_2c20280	hypothetical protein	10.5-		
BGL_2c20290	hypothetical protein	33.1-		2.1-
BGL_2c20300	hypothetical protein	43.6-		
BGL_2c20310	hypothetical protein	7.4-		
BGL_2c20320	2-aminoethylphosphonate-pyruvate transaminase PhnW	3.7-		
BGL_2c20330	O-acetylhomoserine/O-acetylserine sulfhydrylase	4.4-		
BGL_2c20340	fatty acid desaturase type 1	4.3-		
BGL_2c20350	glutamine amidotransferase type 1	4.4-		
BGL_2c20370	glucose-methanol-choline oxidoreductase	3.3-		

Locus_tag	Predicted function	Fold change		
		BGPG2	BGPG3	BGPG4
BGL_2c20530	transcriptional regulator, LysR family	2.3-		
BGL_2c20610	hypothetical protein	3.7-		
BGL_2c20620	ABC transporter, substrate binding protein	3.0-		
BGL_2c20630	ABC transporter, permease protein	2.7-		
BGL_2c20660	putative acetylpolyamine aminohydrolase AphA	2.2-		
BGL_2c20670	putative glycoside hydrolase	7.7-		
BGL_2c20920	glycoside hydrolase family 28	2.7+		
BGL_2c21180	glutamine ABC transporter substrate binding protein			2.3-
BGL_2c21190	glutamine ABC transporter permease protein			2.6-
BGL_2c21770	alpha amylase	2.3-		2.8-
BGL_2c21940	glycosyl transferase			2.1-
BGL_2c22020	Putative transposase of IS4/5 family (DUF4096)	2.1-		
BGL_2c22460	type VI secretion system Vgr family protein	2.4-		
BGL_2c22490	spermidine/putrescine ABC transporter periplasmic spermidine/putrescine-binding protein	2.8-		
BGL_2c22500	spermidine/putrescine ABC transporter permease protein	3.7-		
BGL_2c22570	Putative glucose uptake permease	2.1-		2.0-
BGL_2c22580	hypothetical protein	5.0-		
BGL_2c22590	putative translation initiation inhibitor	5.8-		
BGL_2c22600	asparagine synthase, glutamine-hydrolyzing AsnB	6.2-		
BGL_2c22610	3-phosphoshikimate 1-carboxyvinyltransferase AroA	7.7-		
BGL_2c22620	3-phosphoshikimate 1-carboxyvinyltransferase AroA	7.6-		
BGL_2c22750	aldehyde dehydrogenase			2.3-
BGL_2c22830	hypothetical protein		2.8+	2.3+
BGL_2c23140	ribose transport system permease protein RbsC			2.0-
BGL_2c23230	hypothetical protein	2.5+	2.2+	2.3+
BGL_2c23260	putative lipoprotein	137.3-		3.0-
BGL_2c23270	hypothetical membrane protein	43.8-		
BGL_2c23280	nitrate reductase alpha subunit	2.5+		
BGL_2c23410	thiaminase I precursor	21.9-		
BGL_2c23420	phosphomethylpyrimidine kinase ThiD	21.8-		
BGL_2c23430	methyltransferase type 11	21.6-		
BGL_2c23440	thymidylate synthase ThyA	22.2-		
BGL_2c23450	nucleoside 2-deoxyribosyltransferase	22.9-		
BGL_2c23460	MutT/nudix hydrolase	21.0-		
BGL_2c23470	phosphoethanolamine N-methyltransferase	157.8-		
BGL_2c23480	gamma-butyrobetaine 2-oxoglutarate dioxygenase	104.7-		
BGL_2c23570	hypothetical protein	3.8+		
BGL_2c23940	response regulator receiver protein	71.3-		
BGL_2c23990	acyl-CoA N-acyltransferase			2.3-
BGL_2c24060	fimbrial biogenesis outer membrane usher protein			2.3-
BGL_2c24070	pili assembly chaperone			2.3-

Locus_tag	Predicted function	Fold change		
		BGPG2	BGPG3	BGPG4
BGL_2c24110	glyoxalase/bleomycin resistanceprotein/dioxygenase			2.9-
BGL_2c24130	amylo-alpha-1,6-glucosidase family			2.2-
BGL_2c24160	sensor histidine kinase	2.2+		
BGL_2c24570	hypothetical protein	2.7-	2.8+	2.6+
BGL_2c24900	putative NmrA-like family protein	3.9+		
BGL_2c24910	maleylacetoacetate isomerase MaiA	3.1+		
BGL_2c24930	homogentisate 1,2-dioxygenase HmgA	3.2+		
BGL_2c24950	putative insertion element protein	9.3-		
BGL_2c24960	hypothetical protein, CoA-dependent acyltransferase	10.7-		
BGL_2c24990	putative PAS/PAC sensor signal transduction histidine kinase	2.2-		
BGL_2c25000	putative haem oxygenase-like protein	2.1-		
BGL_2c25210	putative xanthine dehydrogenase yagR molybdenum-binding subunit			2.0-
BGL_2c25430	putative exported protein	2.2-		
BGL_2c25640	putative membrane protein	2.3+		
BGL_2c25680	putative membrane protein DUF1295	2.3-		
BGL_2c25690	cyclopropane-fatty-acyl-phospholipid synthase (cyclopropane fatty acid synthase)	2.7-		2.7-
BGL_2c25700	lipocalin-like protein	2.5-		3.0-
BGL_2c25750	major facilitator superfamily MFS-1 transporter	3.3-		
BGL_2c25760	MlrC domain protein	3.0-		
BGL_2c25770	hypothetical protein	2.9-		
BGL_2c25840	putative flavin-containing monooxygenase	2.0+		
BGL_2c25850	putative short-chain dehydrogenase/reductase SDR	2.8+		
BGL_2c25860	hypothetical protein	2.2+		
BGL_2c26140	hypothetical protein		2.1+	2.2+
BGL_2c26180	putative NAD(P)H dehydrogenase (quinone)	2.3-	2.0+	
BGL_2c26190	putative GCN5-like N-acetyltransferase (GNAT)	3.7-	2.6+	
BGL_2c26210	putative alginate lyase 2	2.9-		
BGL_2c26310	putative glycoside hydrolase family 3 domain protein	2.2-		
BGL_2c26400	hypothetical protein			2.2-
BGL_2c26450	putative prokaryotic membrane lipoprotein			2.0-
BGL_2c26570	putative sugar transporter, major facilitator superfamily (MFS-1) transporter		2.5+	3.6+
BGL_2c26610	putative membrane protein			2.3-
BGL_2c26760	hypothetical protein		3.0+	2.8+
BGL_2c26870	hypothetical protein		2.0+	2.5+
BGL_2c26920	hypothetical protein		3.1+	2.2+
BGL_2c27190	hypothetical protein	3.4-		
BGL_2c27200	putative glutathione-dependent formaldehyde-activating enzyme, GFA	3.1-		

Locus_tag	Predicted function	Fold change		
		BGPG2	BGPG3	BGPG4
BGL_2c27210	putative membrane protein	2.4-		
BGL_2c27220	transcriptional regulator, LysR family	2.1-		
BGL_2c27580	arginine biosynthesis bifunctional protein ArgJ	2.5-		
BGL_2c27610	putative enoyl-CoA hydratase	4.4-		
BGL_2c27620	putative asparagine synthase (glutamine-hydrolyzing)	4.2-		
BGL_2c27630	glutamate 5-kinase	4.0-		
BGL_2c27640	putative transcriptional regulator, LuxR family	41.5-		
BGL_2c27760	putative methyl-accepting chemotaxis sensory transducer		2.5+	2.3+
BGL_2c27900	5-oxoprolinase (ATP-hydrolyzing)	3.8+		
BGL_2c27990	putative gluconolactonase			2.4+
BGL_2c28040	hypothetical protein		2.5+	2.3+
BGL_2c28120	putative natural resistance-associated macrophage protein			3.7+
BGL_2c28180	putative lysine-arginine-ornithine-binding periplasmic protein	2.5+		
BGL_2c28530	phenylacetic acid degradation-like protein	2.3-		
BGL_2c28540	putative alpha-methylacyl-CoA racemase			2.7-
BGL_2c28550	putative AcnD-accessory protein PrpF			2.4-
BGL_2c28560	Fe/S-dependent 2-methylisocitrate dehydratase AcnD family			2.5-
BGL_2c28610	putative diguanylate cyclase/phosphodiesterase with PAS sensor	2.4-		
BGL_2c28620	hypothetical protein	4.5-		
BGL_2c28640	transcriptional regulator, IclR family	2.1-		
BGL_2c28730	outer membrane porin, OprD family		2.4-	2.5-
BGL_2c28750	amino acid/peptide transporter family		2.0-	2.5-
BGL_2c28900	hypothetical protein	153.8-		
BGL_2c28920	hypothetical protein	2.6+	2.0-	
BGL_2c28930	carbon starvation protein CstA	2.5+		
BGL_2c28940	hypothetical protein	2.2+		2.0-
BGL_2c28960	porin Gram-negative type			2.1-
BGL_2c29020	coenzyme PQQ synthesis protein C			2.1-
BGL_2c29030	coenzyme PQQ synthesis protein B			2.1-
BGL_2c29440	putative membrane protein		2.0-	2.7-
BGL_2c29450	aminotransferase class-III	2.6-	3.1-	4.7-
BGL_2c29460	putative peptidase C45, acyl-coenzyme A:6-aminopenicillanic acid acyl-transferase	2.0-	2.4-	2.3-

VII. Abbreviations

A.	*Agrobacterium*
AA	Amino acid
ACP	Acyl carrier protein
AHL	N-acyl-homoserine lactone
AI	Autoinducer
AI-1	Autoinducer 1
AI-2	Autoinducer 2
AI-3	Autoinducer 3
AIP	Autoinducer peptide
Amp	Ampicillin
AT	*Agrobacterium tumefaciens* medium
B.	*Burkholderia*
BGPG1	*Burkholderia glumae* PG1
BGPG2	*Burkholderia glumae* PG2
BGPG3	*Burkholderia glumae* PG3
BGPG4	*Burkholderia glumae* PG4
bidest	Bidistilled
bps	Base pairs
℃	Degree Celsius
C.	*Chromobacterium*
Cm	Chloramphenicol
CV026	*Chromobacterium violaceum* CV026
DH5α	*E. coli* DH5α
DMF	Dimethylformamide
DMSO	Dimethlsulfoxid
DNA	Desoxyribonucleicacid
dNTP	Desoxyribonuleosi-5'-triphosphate
DPD	(*S*)-4, 5-dihydroxy-2, 3-pentanedione
DSF	Diffusible signal factor
E.	*Escherichia*
E. coli	*Escherichia coli*
E-cup	Eppendorf cup
EDTA	Ethylendiamintetraacetate
e.g.	For example
et al.	*et alii* (and others)
EtOH	Ethanol
Gm	Gentamycin
g	Gram
h	hour
H_2O_{bidest}	Double distilled water
HPLC	High performance liquid chromatography

IPTG	Isopropyl-β-D-1-thiogalactopyranoside
k	Kilo
Kan	Kanamycin
kb	Kilo base pairs
kDa	Kilo Dalton
l	Liter
LB	Luria Bertani
M	Mole(mol/L)
mA	Milli Ampere
max.	Maximum
Mbp	Mega base pairs
MCS	Multiple cloning site
MetOH	Methanol
mg	Milli gram
µg	Micro gram
min	Minute
min.	Minimum
ml	Milli liter
mM	Milli mole
mol	Molar
MS	Mass spectrometry
n	Nano
NCBI	National Center for Biotechnology Information
nm	Nanometer
NTL4	*Agrobacterium tumefaciens* NTL4
OD	Optical density
ONPG	*ortho*-nitrophenyl-β-D-galactopyranoside
ORF	Open reading frame
P.	*Pseudomonas*
PCR	Polymerase chain reaction
Pfu	*Pyrococcus furiosus*
PQS	*Pseudomonas* quinolone signal (2-heptyl-3hydroxy-4 quinolone)
QS	Quorum sensing
R	Resistance
RNA	Ribonucleic acid
RNase	Ribonuclease
rpm	Rounds per minute
SAM	*S*-adenosyl methionine
SDS	Sodium-dodecylsulphate
sec	Second
sp.	Species
T1SS	Type I secretion system
T2SS	Type II secretion system
T3SS	Type III secretion system
T4SS	Type IV secretion system

T5SS	Type V secretion system
T6SS	Type VI secretion system
Tann	Annealing temperature
Taq	*Thermus aquaticus*
TE	Tris-EDTA
Tet	Tetracyline
TLC	Thin layer chromatography
Tm	Melting temperature
Tris	Tris-(hydroxymethylene)-aminoethane
TY	TY medium
TZC	Tetrazolium chloride
U	Unit
V	Volt
v/v	Volume per volume
WT	Wilde type
w/v	Weight per volume
X-Gal	5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside

Amino acid abbreviation codes

A	Ala	Alanine	M	Met	Methionine
C	Cys	Cysteine	N	Asn	Asparagine
D	Asp	Aspartic acid	P	Pro	Proline
E	Glu	Glutamic acid	Q	Gln	Glutamine
F	Phe	Phenylalanine	R	Arg	Arginine
G	Gly	Glycine	S	Ser	Serine
H	His	Histidine	T	Thr	Threonine
I	Ile	Isoleucine	V	Val	Valine
K	Lys	Lysine	W	Trp	Tryptophan
L	Leu	Leucine	Y	Tyr	Tyrosine

Declaration

I hereby declare, on oath, that I have written the present dissertation by my own and have not used other than the acknowledged resouces and aids.

Hamburg, _____ Signature: _____