Complete Genome Sequence Data of Four *Burkholderia glumae* Strains Isolated from Rice Fields in the United States

Hyun-Hee Lee,¹ Tiago Lelis,² John Ontoy,² Jobelle Bruno,² Jong Hyun Ham,^{2,†} and Young-Su Seo^{1,†}

¹ Department of Integrated Biological Science, Pusan National University, Busan 46247, Korea

² Department of Plant Pathology and Crop Physiology, Louisiana State University Agricultural Center, Baton Rouge, LA 70803, U.S.A.

Abstract

Bacterial panicle blight caused by *Burkholderia glumae* is a major disease in rice production worldwide. Currently, only a few whole-genome sequences of *B. glumae* strains isolated in the United States are available. Here, we report the complete genome sequence of four *B. glumae* strains, including three virulent strains (336gr-1, 411gr-6, and 957856-41-c) and the nonpathogenic strain *B. glumae* 257sh-1, which were isolated from rice fields in Louisiana (336gr-1, 957856-41-c, and 257sh-1) and Arkansas (411gr-6). The whole-genome sequence data of *B. glumae* strains will contribute to investigations of the molecular mechanism underlying bacterial pathogenicity and virulence to rice plants.

Genome Announcement

Burkholderia glumae was first reported in Japan as a causal agent for seedling blight and grain rotting (Goto and Ohata 1956). In the United States, bacterial panicle blight caused by *B. glumae* is the primary bacterial disease of rice (Ham et al. 2011). Bacterial panicle blight is also a frequent problem in several rice-producing areas worldwide (Cui et al. 2016). The pathogen infection leads to the sterility of the spikelet and discoloration of the emerging grains, affecting the quantity and quality of rice grains (Nandakumar et al. 2009). The virulence of *B. glumae* to rice plants requires numerous virulence factors such as toxoflavin, lipase, type III secretion effectors, catalase, and extracellular protease (Chun et al. 2009; Devescovi et al. 2007; Kim et al. 2004; Lelis et al. 2019). *B. glumae* strains are phenotypically diverse, infecting many plant species ranging from monocot to dicot (Jeong et al. 2003). Strains isolated from symptomatic rice plants showed variation in virulence, toxin, and pigment production (Karki et al. 2012). Some pigment-producing strains of *B. glumae* showed an intense antifungal activity against *Colletotrichum orbiculare* (Karki et al. 2012). Despite the economic impact caused by *B. glumae* on rice production worldwide, only a few genomes of *B. glumae* were fully sequenced and assembled.

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Keywords

bacterial panicle blight, *Burkholderia glumae*, rice

[†]Corresponding authors: J. H. Ham; jham@agcenter.lsu.edu; and Y.-S. Seo; yseo2011@pusan.ac.kr

H.-H. Lee and T. Lelis contributed equally to this work.

Current address for T. Lelis: Tropical Research and Education Center, University of Florida, Homestead, FL 33031, U.S.A.

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Fig. 1. Virulence phenotypes of *Burkholderia glumae* 257sh-1, *B. glumae* 957856-41-c, *B. glumae* 336gr-1, and *B. glumae* 411gr-6. The disease severity index used in this study was based on a 0-to-9 scale, where 0 = no symptoms and 9 = more than 90% of symptomatic area in each panicle. Each data point represents the average value of three replications (one replication = three plants in each pot). Significant differences among the data points were determined at $P < 0.05$ using Tukey's honestly significant difference test. For inoculation, immature rice panicles were sprayed with the bacterial suspension of each strain at the concentration of approximately 5×10^7 CFU/ml (optical density at 600 nm = 0.1). After inoculation, plants were maintained in the greenhouse room until the completion of data collection. Photos were taken at 11 days after bacterial inoculation.

Here, we report the whole-genome sequence data of four *B. glumae* strains showing different phenotypic traits in virulence and metabolism: 257sh-1, 336gr-1, 411gr-6, and 957856-41-c. *B. glumae* 257sh-1 is a natural nonpathogenic and pigment-producing strain from Louisiana (Karki et al. 2012). *B. glumae* 336gr-1 is a virulent and non-pigment-producing strain from Louisiana (Karki et al. 2012). *B. glumae* 957856-41-c was previously reported as a virulent and non-pigment-producing strain from Louisiana (Karki et al. 2012). However, this strain produced a much lesser amount of the major virulence factor, toxoflavin, compared with other virulent strains (Karki 2010). We reevaluated the virulence of *B. glumae* 957856-41-c along with other strains sequenced in this study, in which this strain was almost nonpathogenic showing no significant difference from the nonpathogenic strain, *B. glumae* 257sh-1 (Fig. 1). *B. glumae* 411gr-6 is a highly virulent and pigment-producing strain from Arkansas (Karki et al. 2012). The virulence phenotypes of the four strains sequenced in this study are presented in Figure 1.

Genomic DNA was extracted using the GenElute Bacterial Genomic DNA Extraction kit (Sigma-Aldrich) following manufacturer protocols. NanoDrop ND-100 Spectrophotometer (NanoDrop Technologies, Inc., Wilmington, DE, U.S.A.) was used to assess the quality and quantity of the DNA sample. Whole-genome sequencing library was prepared with a SMRTbell template prep kit (Pacific Biosciences, Menlo Park, CA, U.S.A.). Single-molecule real-time (SMRT) sequencing was conducted on a PacBio RS II platform (Pacific Biosciences) by Macrogen (Seoul, South Korea) for strains 336gr-1, 411gr-6, and 957856-41-c, and ChunLab (Seoul, South Korea) for strain 257sh-1. *De novo* assembly with the hierarchical genome assembly process (HGAP) version 3 was implemented in the PacBio SMRT Analysis algorithm version 2.3.0 package (Chin et al. 2013). The HGAP data processing consisted of PreAssembler for filtering, Celera assembler for assembly, and BLASR for mapping (Chin et al. 2013). The initial assembly was followed by two rounds of polishing by Quiver for further quality improvements of genome sequences (Chin et al. 2013). Gene prediction and annotation were initially performed by Prokka version 1.12 (Seemann 2014). The genome sequence was reannotated with the NCBI prokaryotic genome annotation pipeline (Tatusova

Table 1. Genome features and statistics of the *Burkholderia glumae* genomes

Features	<i>B. glumae</i> strains			
	336gr-1	411gr-6	957856-41-c	257sh-1
Chromosome	2	2	2	2
Plasmid	3	2	2	2
NCBI accession	CP023203-7	CP021157-60	CP021074-7	CP035900-3
N ₅₀	3.60	3.60	3.76	3.54
Coverage	110X	143X	128X	168X
Size (Mb)	6.88	6.75	6.95	6.69
GC (%)	68.12	68.23	68.10	68.19
Protein	5896	5998	6004	5606
Ribosomal RNA	15	15	15	15
Transfer RNA	77	77	78	66

Table 2. Predicted effector proteins secreted by type III (T3) secretion system in the *Burkholderia glumae* genomes

Strains, locus ID	Conserved chaperone binding domain (CCBD) detection by EffectiveCCBD	EffectiveT3 scores
336gr-1		
CG017_RS05450	CCBD (ITPAVSFVVN) found downstream of expected region	1
CG017_RS28175	CCBD (LGRDVLDDVN) found downstream of expected region	1
411gr-6		
B7760_RS15050	CCBD (ITPAVSFVVN) found downstream of expected region	1
B7760_RS27555	CCBD (LGRDVLDDVN) found downstream of expected region	1
957856-41-c		
B7759_RS06100	CCBD (LRESIQRVVN) found downstream of expected region	1
B7759_RS16550	CCBD (ITPAVSFVVN) found downstream of expected region	1
B7759_RS20495	CCBD (LGRDVLDDVN) found downstream of expected region	1
B7759_RS24610	CCBD (IYLPYFVSN) found within expected region	1
257sh-1		
EXE55_RS10890	CCBD (ITPAVSFVVN) found downstream of expected region	1
EXE55_RS22555	CCBD (LGRDVLDDVN) found downstream of expected region	1

et al. 2016) based on the best-placed reference protein set and, finally, deposited in the NCBI database.

The detailed genome features and statistics of four *B. glumae* strains are summarized in Table 1. All *B. glumae* strains (336gr-1, 411gr-6, 957856-41-C, and 257sh-1) harbored two circular chromosomes (size ranging from 3,761,157 to 3,537,953 bp), and two plasmids (size ranging from 193,251 to 135,963 bp), except *B. glumae* 336gr-1, which carried additional plasmid (size 111,135 bp). In addition, the four *B. glumae* genomes were analyzed for putative type III secretion systems (T3SS) and effector proteins of T3SS. The EffectiveDB server was used for identification of the potential T3SS with EffectiveS346, identification of effector proteins with EffectiveT3 for prediction of secretion signals, and conserved chaperone binding domain detection with EffectiveCCBD (Eichinger et al. 2016). All genomes of four *B. glumae* strains encoded genes for T3SS and effector proteins. In particular, Table 2 shows the potential effector proteins which were predicted by both EffectiveT3 and EffectiveCCBD tools (Eichinger et al. 2016). The whole-genome sequence reported here will contribute not only to our understanding of *B. glumae* genomic variation but also to development of strategies to prevent and control bacterial panicle bight caused by *B. glumae*.

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