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Pathogen profile

Burkholderia glumae: next major pathogen of rice?

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SUMMARY

Burkholderia glumae causes bacterial panicle blight of rice, which is an increasingly important disease problem in global rice production. Toxoflavin and lipase are known to be major virulence factors of this pathogen, and their production is dependent on the TofI/TofR quorum-sensing system, which is mediated by *N*-octanoyl homoserine lactone. Flagellar biogenesis and a type III secretion system are also required for full virulence of *B. glumae*. Bacterial panicle blight is thought to be caused by seed-borne *B. glumae*; however, its disease cycle is not fully understood. In spite of its economic importance, neither effective control measures for bacterial panicle blight nor rice varieties showing complete resistance to the disease are currently available. A better understanding of the molecular mechanisms underlying *B. glumae* virulence and of the rice defence mechanisms against the pathogen would lead to the development of better methods of disease control for bacterial panicle blight.

Taxonomy: *Bacteria*; *Proteobacteria*; *Betaproteobacteria*; *Burkholderiales*; *Burkholderiaceae*; *Burkholderia*.

Microbiological properties: Gram-negative, capsulated, motile, lophotrichous flagella, pectolytic.

Disease symptoms: Aborted seed, empty grains as a result of failure of grain filling, brown spots on panicles, seedling rot.

Disease control: Seed sterilization, planting partially resistant lines (no completely resistant line is available).

Known virulence factors: Toxoflavin, lipase, type III effectors.

INTRODUCTION

Burkholderia glumae (formerly *Pseudomonas glumae*) was first described in Japan as causing grain rot, sheath rot and seedling rot, depending on the rice growth stages (Goto and Ohata, 1956; Goto *et al*., 1987; Kurita and Tabei, 1967; Uematsu *et al*., 1976). Since then, *B. glumae* has also been reported as a rice pathogen from other rice-growing countries in East Asia (Chien and Chang, 1987; Cottyn *et al*., 1996a, b; Jeong *et al*., 2003; Luo *et al*., 2007; Trung *et al*., 1993) and Latin America (Nandakumar *et al*., 2007b; Zeigler and Alvarez, 1989). In the USA, *B. glumae* was identified as the major causal agent of bacterial panicle blight (Nandakumar *et al*., 2005, 2009; Shahjahan *et al*., 2000). Another *Burkholderia* species, *B. gladioli*, also causes grain rot and seedling rot (Ura *et al*., 2006) and bacterial panicle blight (Nandakumar *et al*., 2009) in rice; however, this bacterium tends to be isolated less frequently from infected rice plants and shows less virulence compared with *B. glumae*. According to a recent survey, *B. gladioli* appeared to cause about 20% of bacterial panicle blight occurring in Louisiana and neighbouring rice-growing states, including Texas and Arkansas (A. K. M. Shahjahan and M. C. Rush, unpublished data). Currently, both 'bacterial grain rot' and 'bacterial panicle blight' are used to refer to the rice disease caused by *B. glumae*. The former is widely accepted in Asian countries, whereas the latter is commonly used in the USA and in Latin American countries.

The yield reduction of rice from bacterial panicle blight can reach 75% in severely infested fields as a result of a reduction in grain weight, sterility of florets, inhibition of seed germination and reduction of stands; the year-to-year transmission resulting from the seed-borne nature of the pathogen may also contribute to yield losses (Trung *et al*., 1993). In the southern USA, yield losses caused by outbreaks of bacterial panicle blight in some rice fields in Louisiana were as much as 40% in 1995 and 1998; significant losses caused by this disease were also experienced in 2000 (Nandakumar *et al*., 2009; Shahjahan *et al*., 2000).

Prolonged hot and humid conditions during the rice-growing season favour the development of serious epidemics of bacterial panicle blight. Because the optimal temperature range for the growth of *B. glumae* is relatively high (30–35 °C) (Kurita *et al*., 1964), it is believed that this disease may occur more frequently in tropical and semi-tropical countries and during growing seasons with higher than normal temperatures. Current global climate change may cause an increase in new or previously negligible diseases. Indeed, numerous plant pathogens with high optimal temperatures have emerged or become prevalent

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worldwide (Schaad, 2008). In this sense, the rice disease caused by *B. glumae* should be recognized as a potential threat to the world's rice production; increasing reports of this disease from many rice-growing countries strongly support this notion.

In this article, we present an overview of the various aspects of *B. glumae* and the rice disease that it causes. In particular, recent studies on the molecular biology and molecular genetics underlying the bacterial pathogenesis by *B. glumae* are described comprehensively.

TAXONOMY, PHYSIOLOGICAL PROPERTIES AND HOST RANGES OF *B. GLUMAE*

The genus *Burkholderia*

In 1992, seven species of *Pseudomonas* in the *Pseudomonas* rRNA homology group II were reclassified as the new genus *Burkholderia* (Yabuuchi *et al*., 1992). The type strain, *Burkholderia cepacia*, was first identified as a plant pathogen causing sour skin disease in onion (Burkholder, 1950). The two rice pathogenic bacteria, *Pseudomonas glumae* and *P. plantarii*, were additionally transferred from the genus *Pseudomonas* to the genus *Burkholderia* in 1994 (Urakami *et al*., 1994). Since then, the genus *Burkholderia* has grown considerably with the transfer of other bacterial species to the genus and with the identification of new species in the genus. In 2003, there were over 30 species in the genus; in 2008, over 40 described species were listed in the genus (Coenye and Vandamme, 2003; Compant *et al*., 2008). Currently, over 60 species are listed in the genus *Burkholderia* (http://www.bacterio.cict.fr/b/burkholderia.html). *Burkholderia glumae* is one of several plant pathogenic members of the genus, which includes species pathogenic to humans and animals, and species with nonpathogenic and even beneficial associations with plants (Coenye and Vandamme, 2003; Compant *et al*., 2008; Parke and Gurian-Sherman, 2001).

Burkholderia glumae

Burkholderia glumae is Gram-negative, aerobic and motile with two to four polar flagella; it is rod shaped, with a size range of 0.5–0.7 × 1.5–2.5 μm in width and length, respectively; it is able to grow at 11–40 °C (optimally at 30–35 °C) and is estimated to have total DNA with 68.2% GC content (Fig. 1C) (Brenner *et al*., 2005; Schaad *et al*., 2001). The pathogen can be isolated and identified by its morphological characteristics on the semi-selective medium, sucrose–phosphate–glutamate (S-PG), but can be grown on a number of laboratory media (Schaad *et al*., 2001; Tsuchima *et al*., 1986).

Several molecular biology tools and techniques, including polymerase chain reaction (PCR), reverse transcriptase-PCR (RT-PCR) and rep-PCR, have been developed or are being used to identify *B. glumae* and to detect its presence in plant tissues. Primers specific for the detection of the closely related species *B. plantarii* and *B. glumae* have been developed from the 16S-23S rDNA spacer region for use in conventional PCR (Takeuchi *et al*., 1997). Real-time PCR detection methods have been developed to detect the presence of *B. glumae* in rice seed and to quantify the amount of *B. glumae* present in seeds (Nandakumar *et al*., 2009; Sayler *et al*., 2006). Rep-PCR fingerprint analysis has been proven to have great ability to detect differences among *B. glumae* strains from within a geographical location, as 22 different fingerprints have been detected for 25 different strains (Sayler *et al*., 2006).

Previous studies have used 16S-23S rDNA internal transcribed spacer (ITS) sequences to analyse the genetic diversity of *B. glumae* strains isolated from the same geographical location and from different geographical locations (Sayler *et al*., 2006; Takeuchi *et al*., 1997). ITS sequence analysis of six *B. glumae* strains isolated from rice in Arkansas showed greater than 99.5% similarity when compared with each other and with a single isolate from Japan (Sayler *et al*., 2006). The comparison of 20 strains from different geographical regions in Japan showed that all *B. glumae* strains had identical ITS regions (Takeuchi *et al*., 1997).

The DNA sequences of a number of genes have been utilized to analyse the genetic relatedness of different species within the genus *Burkholderia*, as well as that of different strains within the species *B. glumae*. Recent phylogenetic analyses based on the nucleotide sequences of *rpoB*, *gyrB* and *rrs* (Tayeb *et al*., 2008), *recA* (Payne *et al*., 2005), and *rpoD* and *gyrB* (Maeda *et al*., 2006) have indicated that *B. glumae* is closely related to other rice pathogenic species, *B. plantarii* and *B. gladioli*, but relatively distant from the *B. cepacia* complex, which includes at least four species of plant pathogens infecting onion (Jacobs *et al*., 2008). According to the studies of Payne *et al*. (2005) and Tayeb *et al*. (2008), the *B. cepacia* complex is closer to the animal pathogenic species, *B. mallei* and *B. pseudomallei*, than to *B. glumae*. The *gyrB* and *rpoD* gene sequences have also been used to study the phylogeny of strains within a *Burkholderia* species, and the concatenated sequences of the two genes allowed for the separation of the species within the constructed phylogenetic tree (Maeda *et al*., 2006). In addition, the *gyrB* sequence was used to successfully develop primers able to distinguish between *B. glumae*, *B. gladioli* and *B. plantarii* in a multiplex PCR (Maeda *et al*., 2006). Nucleotide sequence comparisons from strains largely isolated from Japan showed that all *B. glumae* strains were identical in their *gyrB* sequences; sequence comparisons of the *rpoD* gene showed only one or two nucleotide differences in a small number of *B. glumae* strains from Japan and Indonesia (Maeda *et al*., 2006).

The host range of *B. glumae* may not be limited to rice. Jeong *et al*. (2003) reported that *B. glumae* infected other crops, including pepper, eggplant, sesame and tomato, causing bacterial wilt.

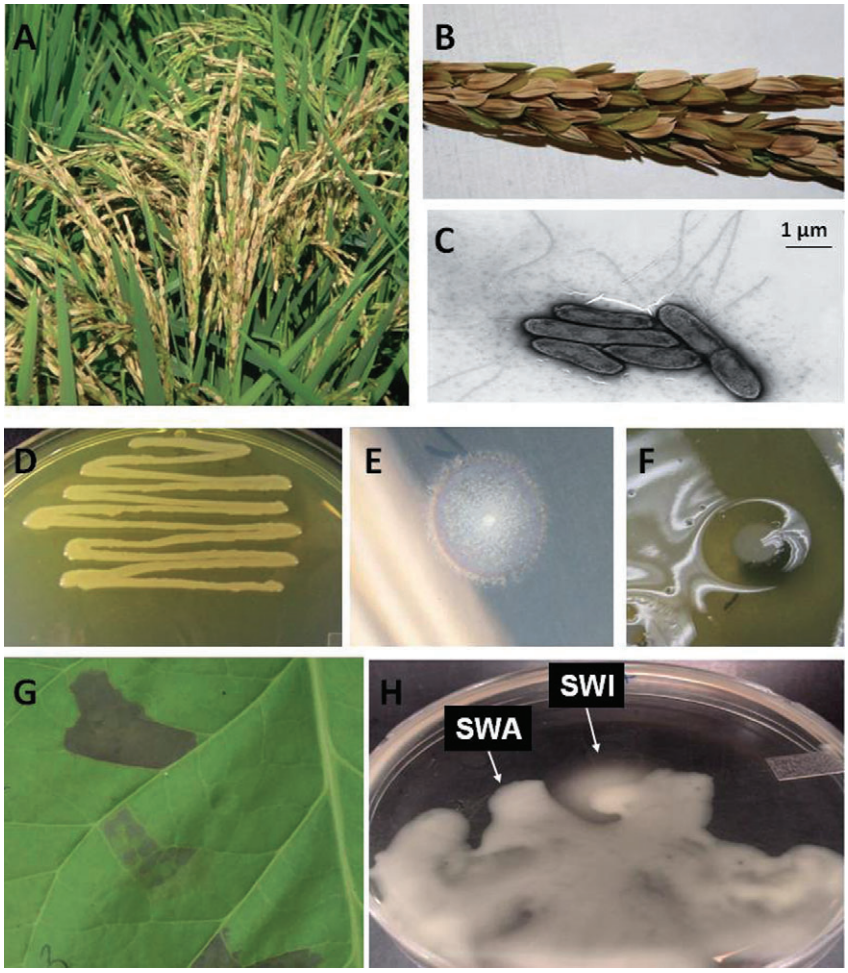

Fig. 1 Symptoms and virulence factors produced by *Burkholderia glumae*: (A, B) typical symptoms of bacterial panicle blight on rice panicles; (C) transmission electron micrograph of *B. glumae* cells and flagella; (D) toxin (toxoflavin) production by *B. glumae* indicated by the yellow pigment on a King's B agar plate; (E) lipase activity indicated by the opaque and iridescent halo around a *B. glumae* colony on a Luria–Bertani (LB) agar plate containing 0.2% Tween-20; (F) pectinase activity indicated by the pitting zone around a *B. glumae* colony on a pectate semi-solid agar plate (Kelemu and Collmer, 1993); (G) hypersensitive reaction (HR) on a tobacco leaf infiltrated with approximately 10^8 colony-forming units (cfu)/mL of *B. glumae*; (H) swimming (SWI) and swarming (SWA) motilities indicated by the round and wavy bacterial zones, respectively, around a *B. glumae* colony on an LB with 0.3% agar plate.

Remarkably, *B. glumae* was isolated from an infant with chronic granulomatous disease, indicating that at least some strains of this pathogen can be opportunistic human pathogens (Weinberg *et al*., 2007). Several other *Burkholderia* spp. are known to contain both animal and plant pathogenic strains within a species. *Burkholderia cenocepacia* includes both clinical and plant pathogenic strains (Gonzalez *et al*., 1997; Jacobs *et al*., 2008; Springman *et al*., 2009), and some clinical strains can cause onion maceration (Springman *et al*., 2009). Likewise, *B. gladioli*, which causes bacterial panicle blight in rice, like *B. glumae*, has been isolated from human patients (Kennedy *et al*., 2007). However, genetic distinction between plant and animal/human pathogenic strains within a *Burkholderia* species is not clear, and it is very probable that some environmental or plant pathogenic strains can also infect humans and animals (Jacobs *et al*., 2008; Springman *et al*., 2009). Although it is still unknown whether the *B. glumae* strains isolated from rice or other field crops can be pathogenic to humans, the clinical strain, AU6208, has been shown to be strongly pathogenic to rice (Devescovi *et al*., 2007). Thus, it is imperative to comprehensively study the genotypic relatedness between 'field' and 'clinical' strains and to evaluate the potential harm of the strains from rice fields to human health.

In 2009, the complete genome sequence of *B. glumae* strain BGR1, isolated from a diseased rice panicle in Korea, was announced (Lim *et al*., 2009). The *B. glumae* strain 336gr-1 used for molecular studies in our laboratory is currently being sequenced. Whole genome sequence information, which can be efficiently generated using recently developed high-throughput DNA sequencing technologies, would provide great opportunities in the study of the population genomics of the species and answer those questions regarding the possible threats to human health.

SYMPTOMS OF BACTERIAL PANICLE BLIGHT

Symptoms of bacterial panicle blight include panicle blight, seedling blight and sheath rot (Nandakumar *et al*., 2009). A linear lesion extending downwards from the leaf blade collar forms on the flag leaf sheath and may be several inches in length; this

lesion has a reddish-brown border and a centre that becomes grey and necrotic. Affected panicles may have one or all of their florets blighted with grains not filling or aborting; the basal third of the florets will begin as a white or light grey colour separated by a reddish-brown margin from the rest of the floret, which eventually becomes straw coloured (Saichuk, 2009). Upright brown panicles caused by the failure of grain filling are typically observed in severely infected fields (Fig. 1A,B).

Several other bacterial species have also been reported to cause similar symptoms. Bacterial stripe disease with grain discoloration has been reported to be caused by *Acidovorax avenae* (formerly *Pseudomonas avenae*) (Kadota and Ohuchi, 1983). In addition, sheath rot and grain discoloration in Latin America are believed to be caused by *Pseudomonas fuscovaginae* as well as *B. glumae* (Zeigler and Alvarez, 1987, 1989). Accurate identification of the pathogens and epidemiological surveys are therefore essential for choosing appropriate control schemes.

EPIDEMIOLOGY

Burkholderia glumae is considered to be a seed-borne pathogen. Hikichi *et al*. (1993) detected *B. glumae* cells in various parts of naturally infected seeds, including the epidermis and parenchyma. In addition, the *B. glumae* cells present on leaf sheaths are essential for primary infection, which, in turn, provide a major source of inoculum to emerging panicles (Tsuchima and Naito, 1991; Tsuchima *et al*., 1996). It has also been observed that visible symptoms always appear first on the first flag leaf sheath and then on panicles when the pathogen is injected into boots (Yuan, 2005). Tsuchima *et al*. (1996) reported that symptoms on flag leaf sheaths forecast the disease on panicles, because flag leaf sheaths are close to panicles and their infection primarily occurs at the heading stage.

Outbreaks of bacterial panicle blight tend to occur under conditions of unusually high temperature, especially at night, and frequent rains (Cha *et al*., 2001). Severe outbreaks of bacterial panicle blight of rice were experienced in 1995 and 1998 in Louisiana and in neighbouring states of the southern USA, causing as much as 40% yield losses in some fields (Shahjahan *et al*., 2000). Record high temperatures were recorded during these seasons, with high temperatures extending into the night. As mentioned previously, heading and flowering stages are the most vulnerable period for bacterial panicle blight; prolonged high temperatures and frequent rainfall during this period are extremely important environmental predispositions for epidemics of this disease (Cha *et al*., 2001; Tsuchima *et al*., 1995). Several disease-forecasting models for bacterial panicle blight have been developed based on various factors during the rice heading period, including the presence of the pathogen in flag leaf sheaths (Tsuchima *et al*., 1996), severely infected panicles at early heading stages (Tsuchima *et al*., 1995) and micro-weather conditions (Lee *et al*., 2004).

In addition, Tsuchima and Naito (1991) have demonstrated that the distribution and spatial patterns of bacterial panicle blight observed in rice paddy fields indicate that severely diseased panicles are important primary inoculum sources, forming infection foci in the fields, and that focal formation is closely related to the early occurrence and disease severity of severely diseased panicles.

CONTROL

Currently, there are few control methods available for bacterial panicle blight of rice (Saichuk, 2009; Sayler *et al*., 2006). The use of pathogen-free seed, however, is recommended to reduce the incidence of bacterial panicle blight (Saichuk, 2009). In this section, other control measures that have been studied are introduced.

Chemical control

Seed treatments and foliar sprays of oxolinic acid, a quinoline derivative, are highly effective for the control of this rice disease (Hikichi, 1993a, b, c). A study of the efficacy of oxolinic acid, with fluorescein isothiocyanate (FITC)-conjugated antibody and fluorescence microscopy, demonstrated that only 3% of seedlings expressed measurable symptoms after treatment, compared with 92% of seedlings from untreated seeds (Hikichi *et al*., 1995). DNA gyrase, composed of GyrA and GyrB, is known to be a major target of oxolinic acid in Gram-negative bacteria, including *B. glumae* (Drlica and Zhao, 1997). However, oxolinic acid is not commercially available in many countries, including the USA. In addition, the occurrence of strains naturally resistant to oxolinic acid is a problem in adopting this chemical to control bacterial panicle blight. Recently, Maeda *et al*. (2004) have reported that the natural occurrence of oxolinic acid-resistant *B. glumae* strains is a result of missense mutations in *gyrA* causing the substitution of the amino acid at position 83 from serine to either arginine or isoleucine. The same research group later reported that the substitution at amino acid position 83 of GyrA also increased the parasitic fitness in the rice spikelet in the presence of oxolinic acid, supporting the notion that the substitution is responsible for the resistance of *B. glumae* field isolates to oxolinic acid (Maeda *et al*., 2007).

Biological control

Several studies have demonstrated that some avirulent strains of *Burkholderia* spp. can suppress the development of bacterial panicle blight, suggesting the possibility of their use as a biological control agent. An avirulent *B. gladioli* strain prevented

the occurrence of the disease almost completely when it was co-inoculated with *B. glumae* on rice panicles (Miyagawa and Takaya, 2000). Rice seedling rot was also suppressed when rice seeds were treated with avirulent strains of *B. glumae* (Furuya *et al*., 1991). In addition to avirulent *Burkholderia* spp., many potential biological control agents showing high levels of antibiotic activity against *B. glumae in vitro* have been isolated from rice fields, including *Bacillus* sp., *Pseudomonas fluorescens* and *Saccharomyces* sp. (M. C. Rush, unpublished data). However, the efficacy of these potential agents for disease control in the field remains to be evaluated.

Resistant cultivars and lines

Varying levels of susceptibility and resistance to bacterial panicle blight exist among rice varieties (Saichuk, 2009; Sayler *et al*., 2006). Some varieties have demonstrated partial resistance to bacterial panicle blight; however, no complete resistance has been revealed from inoculation tests of 100 rice varieties (Shahjahan *et al*., 2000). Among the partially resistant varieties identified, one variety, Jupiter, exhibited a significantly higher level of disease resistance compared with other varieties (Sha *et al*., 2006). In addition, one mutant line, LM-1, generated by gamma radiation of the susceptible cultivar, Lemont, showed significantly higher resistance to bacterial panicle blight (Groth *et al*., 2007; Sayler *et al*., 2006). This partially resistant variety and line are being used to study the genes and molecular mechanisms underlying partial disease resistance to bacterial panicle blight (Nandakumar and Rush, 2008; Nandakumar *et al*., 2007a).

VIRULENCE FACTORS AND THEIR REGULATION

Toxoflavin and lipase are currently known to be the pathogenic determinants of *B. glumae*. Toxoflavin-deficient and lipase-deficient mutant strains are almost avirulent to rice (Devescovi *et al*., 2007; Kim *et al*., 2004). The production of these two pathogenic determinants is dependent on quorum sensing; thus, impairment of the quorum-sensing system of *B. glumae* also results in a loss of virulence (Devescovi *et al*., 2007; Kim *et al*., 2004). Quorum sensing, which causes bacterial genes to be expressed only when the bacterial population density reaches a certain level, is a well-known mechanism of bacterial cell-to-cell communication (Fuqua *et al*., 1996). *N*-Acyl homoserine lactones (AHLs) are common quorum-sensing signals that are utilized by more than 50 species of prokaryotes (Von Bodmann *et al*., 2003). In plant pathogenic bacteria, the production of virulence factors, such as extracellular polysaccharides (EPSs), degradative enzymes and components for Ti plasmid transfer is controlled by AHL quorum sensing (Von Bodmann *et al*., 2003). In *B. glumae*, quorum sensing is mediated by the AHL molecule, *N*-octanoyl homoserine lactone (C8-HSL), and controls a diverse array of cellular processes in addition to the production of toxoflavin and lipase (Chun *et al*., 2009; Kim *et al*., 2007; H. S. Karki and J. H. Ham, unpublished data). For example, flagellar biosynthesis and catalase activity are also regulated by the *B. glumae* quorum-sensing system through an IclR-type transcriptional regulator, QsmR (Chun *et al*., 2009; Kim *et al*., 2007). In addition, Goo *et al*. (2010) have recently performed a proteomic analysis comparing a wild-type and a quorum-sensing-deficient mutant, and demonstrated a complete inventory of *B. glumae* proteins that are produced in a quorum-sensing-dependent manner. In that study, numerous proteins involved in various cellular functions, such as antioxidation and cell attachment, were newly found to be quorum-sensing-dependent gene products in addition to previously known proteins. *Burkholderia glumae* also possesses other virulence factors commonly found in many plant pathogenic bacteria, such as the type III secretion system (TTSS), polygalacturonases and EPSs.

Toxoflavin

Toxoflavin, fervenulin and reumycin are phytotoxins produced by *B. glumae* (Fig. 1D) (Kim *et al*., 2004; Sato *et al*., 1989; Suzuki *et al*., 2004). These toxins are actually very similar to each other in molecular structure (Suzuki *et al*., 2004); however, toxoflavin is most toxic to rice (Sato *et al*., 1989). Suzuki *et al*. (2004) identified and further characterized a polycistronic operon composed of five genes, *toxABCDE*, which encodes components of the toxoflavin biosynthesis pathway. Mutations in this operon resulted in the loss of ability to produce any toxoflavin, fervenulin or reumycin, indicating that these phytotoxins are all dependent on the toxoflavin biosynthesis pathway (Kim *et al*., 2004; Suzuki *et al*., 2004).

Toxoflavin, an azapteridine antibiotic, was first isolated from *B. cocovenenans*, and its molecular structure was determined in the early 1960s (Levenberg and Linton, 1966). This toxin reduces the elongation of sprouts and roots of rice seedlings (Suzuki *et al*., 1998; Yoneyama *et al*., 1998) and causes a characteristic symptom of rice grain rot on panicles (Iiyama *et al*., 1995). Jeong *et al*. (2003) showed that toxoflavin also induced bacterial wilt on a number of crops other than rice. Toxoflavin was also detected in several strains of *B. gladioli* (Suzuki *et al*., 1998) and from rice seedlings infected with *B. glumae* (Iiyama *et al*., 1995).

Toxoflavin is considered to be an important virulence factor of *B. glumae* because the toxin is able to induce symptoms on rice and because toxoflavin-deficient strains show almost avirulent phenotypes (Kim *et al*., 2004; Nandakumar *et al*., 2009; Wang *et al*., 1991; Yoneyama *et al*., 1998). However, another study has revealed that *B. glumae* mutants disrupted in the toxoflavin biosynthesis genes are as virulent in rice seedlings as their parental strain, and are still able to induce symptoms on rice

panicles even though these symptoms are significantly less than those caused by the parental strain (Suzuki *et al*., 2004). We also observed that a *toxA*⁻ derivative, impaired in the toxoflavin biosynthesis pathway, was much less virulent than the parental strain, but still induced necrotic symptoms on the panicles of bacterial panicle blight-susceptible cultivars (B. Shrestha and J. H. Ham, unpublished data). These observations indicate that, although the phytotoxins represented by toxoflavin are important virulence factors, the pathogenesis of *B. glumae* is a complicated process that involves multiple virulence factors.

Several recent studies have described the regulatory elements controlling toxoflavin biosynthesis (Kim *et al*., 2004; Shingu and Yoneyama, 2004; Suzuki *et al*., 2004). An open reading frame, designated *toxR*, was found downstream of the *toxABCDE* operon and shared the same transcriptional orientation (Suzuki *et al*., 2004). *toxR*, which encodes a LysR-type transcriptional activator, was absolutely required for toxoflavin biosynthesis, because *B. glumae* mutants with a disrupted *toxR* gene could not produce detectable amounts of toxoflavin (Suzuki *et al*., 2004). Suzuki *et al*. (2004) proposed a biosynthesis pathway for toxoflavin, modelled after the biosynthesis pathway for riboflavin in bacteria, which consists of five enzymatic reactions, beginning with the guanosine 5′-triphosphate (GTP). The product of the *toxR* gene was later concluded to be a transcriptional regulator that binds to the promoter region of *toxA* and presumably is responsible for transcriptional activation of the *tox* operon (Shingu and Yoneyama, 2004). Further studies by Kim *et al*. (2004) revealed additional important facts related to toxoflavin biosynthesis and transport. Among these are the presence of a second polycistronic operon, *toxFGHI*, composed of genes responsible for toxoflavin transport; the activity of toxoflavin as a co-inducer for the product of *toxR*; and the presence of an additional gene, *toxJ*, coding for another transcriptional activator of toxoflavin biosynthesis (Kim *et al*., 2004).

In particular, Kim *et al*. (2004) demonstrated the importance of the *B. glumae* quorum-sensing system in the regulation of toxoflavin biosynthesis, and identified the genes for the quorum-sensing system, *tofI* and *tofR*, which encode the AHL synthase for *N*-hexanoyl homoserine lactone (C6-HSL) and C8-HSL and a cognate receptor for C8-HSL, respectively. In the same report, they also proposed a model for the regulatory cascade leading to the expression of the toxoflavin biosynthesis genes, in which the TofI/TofR quorum-sensing system regulates the expression of *toxJ*; ToxJ, in turn, activates the transcription of *toxR*, encoding a LysR-type transcriptional activator; and both ToxJ and ToxR activate the expression of the genes for the biosynthesis and transport of toxoflavin (Kim *et al*., 2004). Toxoflavin production therefore apparently does not begin until *B. glumae* populations reach high levels as detected by quorum sensing.

Recently, the phototoxicity of toxoflavin was applied to the development of a new selection marker system for plant transformation. Koh *et al*. (2010) identified a gene, *tflA*, encoding a toxoflavin-degrading enzyme from the JH2 strain of the bacterium *Paenibacillus polymyxa* and utilized this gene as a selection marker for transformed plants in toxoflavin-containing media.

Lipase

Burkholderia glumae has been commercially utilized because of its strong ability to produce lipase (Fig. 1E), which is used for many industrial purposes, including detergent production (Boekema *et al*., 2007; Frenken *et al*., 1993; Rosenau and Jaeger, 2000). Recently, Devescovi *et al*. (2007) reported that the clinical strain of *B. glumae*, AU6208, isolated from an infant patient with granulomatous disease, was highly pathogenic to rice. In the same study, a derivative of AU6208 defective in *lipA*, which encodes the LipA lipase, was much less virulent to rice than the parental strain, indicating that lipase is an important virulence factor for the pathogenesis of the strain in rice. In our study, mutation of the type II secretion system resulted in the reduced activity of lipase in the medium, indicating that lipase is secreted through the type II secretion pathway (H. S. Karki and J. H. Ham, unpublished data). Kang *et al*. (2008) found, through a proteomics analysis, that at least 16 extracellular proteins were secreted by *B. glumae* via its type II secretion pathway. They revealed, in the same study, that a *B. glumae* mutant defective in the type II secretion system was much less virulent than its virulent parent. However, the virulence functions of the secreted proteins, with the exception of lipase, are still unknown (Kang *et al*., 2008).

According to Devescovi *et al*. (2007), lipase production, like toxoflavin biosynthesis, by *B. glumae* is dependent on the TofI/TofR quorum-sensing system mediated by C8-HSL (Devescovi *et al*., 2007). Other virulence factors controlling the lipase production of *B. glumae* have not yet been reported.

Flagella-dependent motility

Although bacterial flagella do not have direct detrimental effects on host plants, the motility mediated by them is often very important for bacterial pathogenesis (Hase, 2001; Liao *et al*., 2009; Martinez *et al*., 2010; McNally *et al*., 2007). Kim *et al*. (2007) recently observed that the polar flagella of *B. glumae* are responsible for at least two types of bacterial motility, swimming and swarming, on Luria–Bertani (LB) agar plates with 0.7% and 0.4% agar, respectively, and that all *B. glumae* mutants defective in flagellar biogenesis, and thus showing nonmotile phenotypes, are almost avirulent to rice, suggesting that flagella-mediated motility is an essential part of pathogenesis by *B. glumae*. Figure 1H shows both swimming and swarming activities of *B. glumae* on LB medium containing 0.3% agar.

Like toxoflavin biosynthesis and lipase production, flagellar biosynthesis and the motility driven by it have been shown to be

dependent on the TofI/TofR quorum-sensing system mediated by the C8-HSL signal (Kim *et al*., 2007). In the same study, additional regulatory factors governing motility were discovered, including an IclR-type transcriptional regulator, QsmR, and FlhD/FlhC. In addition, a working model was proposed for the regulatory cascade controlling flagellar biosynthesis based on observations of the following sequential transcriptional activation: active form of TofR bound to C8-HSL → *qsmR* → *flhDC* → genes for flagellar biosynthesis, chemotaxis and motor functions (Kim *et al*., 2007).

Type III effectors

T3SSs are utilized by many Gram-negative bacterial pathogens for direct translocation of proteinaceous virulence factors, type III effectors, into eukaryotic host cells (Galan and Wolf-Watz, 2006). In many Gram-negative plant pathogenic bacteria, T3SSs are required for both pathogenicity on susceptible host plants and the elicitation of hypersensitive responses (HRs) on the leaves of nonhost plants or resistant host plants (Alfano and Collmer, 2004; Bent and Mackey, 2007). Because of these dual phenotypes, many genes encoding the T3SSs of plant pathogenic bacteria were originally named '*hrp*', which stands for *h*ypersensitive *r*esponse and *p*athogenicity (Alfano and Collmer, 2004).

Kang *et al*. (2008) reported that *B. glumae* could elicit HRs on tobacco leaves in a T3SS-dependent manner, like other plant pathogenic bacteria that possess T3SSs (Fig. 1G). Recently, Kang *et al*. (2008) characterized the gene cluster encoding the T3SS of *B. glumae* and identified up to 28 extracellular proteins which were accumulated in an HrpB-dependent manner. HrpB is a key regulatory factor for the expression of the genes for T3SS and type III effectors of a related bacterium, *Ralstonia solanacearum* (Van Gijsegem *et al*., 1995). All 28 extracellular proteins, however, were also secreted by a T3SS-deficient mutant, indicating that they were not secreted in a T3SS-dependent manner (Kang *et al*., 2008); nevertheless, the T3SS-deficient mutant showed significantly less virulence on rice panicles compared with the parental strain. Although this implies that type III effectors are collectively required for the full virulence of *B. glumae*, type III effectors secreted via the *B. glumae* T3SS have not yet been reported.

EPSs

EPSs play vital roles in the pathogenesis of vascular plant pathogens that colonize xylem vessels (Denny, 1995). In general, EPSs produced by bacterial pathogens cause the occlusion of the host vascular system leading to the development of wilting. Like the *hrp* genes for T3SS components, the genes involved in EPS biosynthesis are clustered in a genomic region. EPS production is a primary virulence factor of several important plant pathogenic bacteria, including *Ralstonia solanacearum*, *Erwinia amylovora*, *Pantoea stewartii* ssp. *stewartii* and *Xanthomonas campestris* (Denny, 1995). The role of EPSs in bacterial panicle blight of rice has not been reported. However, Jeong *et al*. (2003) reported that *B. glumae* caused wilting symptoms in various field crops, including sesame, pepper and eggplant; *B. glumae* also produces a visible amount of EPSs on 'CPG' (casamino acid peptone glucose) agar medium (Schaad *et al*., 2001). In light of the pivotal roles of EPSs in other plant diseases, EPSs produced by *B. glumae* should be considered as important potential virulence factors contributing to bacterial panicle blight in rice.

Polygalacturonases

Pectic enzymes, which degrade pectic polymers, are important virulence factors for many plant pathogenic bacteria, including soft rot-causing bacteria (Abbott and Boraston, 2008; Huang and Allen, 1997; Roper *et al*., 2007; Wang *et al*., 2008). In *Burkholderia*, the ability to macerate onion tissue is dependent on the presence of a functional *pehA* gene encoding endopolygalacturonase in plant pathogenic *B. cepacia* (Gonzalez *et al*., 1997) and *B. cenocepacia* (Springman *et al*., 2009), indicating that *pehA* is an important virulence factor of these plant pathogenic *Burkholderia* spp. Interestingly, the *pehA* endopolygalacturonase gene is also highly associated with a greater virulence of *B. cenocepacia* on the nematode *Caenorhabditis elegans* (Springman *et al*., 2009). Recently, *pehA* and *pehB*, which encode two isoforms of endopolygalacturonase, were discovered from the *B. glumae* genome; however, their functions in virulence were not confirmed by mutational analysis of each gene because of a probable functional redundancy between the two isozymes (Fig. 1F) (Degrassi *et al*., 2008).

Molecular genetic studies have largely contributed to the identification and characterization of *B. glumae* genes that encode virulence factors and their regulatory components. Genetic manipulation of *Burkholderia* spp., however, is relatively difficult because of multidrug resistance and recalcitrance to artificial gene transformation. In the case of *B. glumae*, triparental mating is the only reliable method for gene transformation, and marker exchange mutagenesis through double homologous recombination has mostly been used for targeted gene mutation (I. Hwang, Seoul National University, Seoul, Korea, personal communication). Improved methods of electrotransformation and of targeted gene deletion without antibiotic markers have been developed for *B. cenocepacia* strains (Dubarry *et al*., 2010; Flannagan *et al*., 2008). We expect that these new methodologies for gene manipulation will be applied to the *B. glumae* system and will facilitate molecular genetic research to learn more about the virulence system of this pathogen. As increased information

concerning *B. glumae* becomes available, it is likely that additional virulence factors and their regulatory systems and mechanisms involved in pathogenicity will be identified.

CONCLUDING REMARKS AND FUTURE PERSPECTIVES

Bacterial panicle blight is currently widespread around the world and is very likely, because of global warming, to be the next major disease of rice in the near future. Because a severe outbreak of this pathogen would result in devastating damage to grain yield, special efforts should be made to develop accurate disease forecasting systems and efficient control methods. To achieve these goals, a better understanding of *B. glumae* epidemiology and virulence mechanisms, and of host resistance systems, is essential.

Significant advancement in understanding the virulence mechanism of *B. glumae* has recently been made by several leading laboratories. In particular, discoveries of important virulence factors, including toxoflavin and lipase, and of the TofI/TofR quorum-sensing system regulating the production of these virulence factors, have been achieved by molecular genetic and biochemical studies. Nevertheless, our knowledge of this pathogen is still primitive compared with that of other major plant pathogenic bacteria. For example, little information is available on the *B. glumae* regulatory systems for virulence factors other than quorum sensing. Genetic approaches, such as random mutagenesis with a transposon, would be useful to identify additional virulence factors and their regulatory elements. Likewise, our knowledge on rice resistance to bacterial panicle blight is also very limited. No gene-for-gene resistance has been identified for bacterial panicle blight, and the mechanisms for the observed partial resistance to the disease remain to be elucidated. Genetic mapping of the loci conferring partial resistance to bacterial panicle blight would be useful for marker-assisted breeding for disease-resistant lines and to identify candidate resistance gene(s). Physiological defence responses associated with partial resistance to bacterial panicle blight would also provide insight into the biochemical nature of the resistance, as well as its difference from the gene-for-gene resistance for defence.

In addition to the conventional genetic and physiological approaches, genomic studies with increasing whole genome sequence data are expected to be powerful tools to enhance our knowledge on both bacterial virulence and host resistance. Currently available new high-throughput DNA sequencing technologies, such as the Solexa (Illumina Inc., San Diego, CA, USA) and 454 (454 Life Sciences, Branford, CT, USA) systems, and accompanying improved bioinformatics tools, will make genomic studies on rice disease resistance to bacterial panicle blight and bacterial pathogenesis of *B. glumae* more feasible and affordable.

Genome-wide comparison between *B. glumae* and other *Burkholderia* spp. and, further, other plant pathogenic bacteria, would elucidate the genetic elements determining the different ecological niches and pathogenic behaviour of different *Burkholderia* spp. Virulence factors present in both plant and animal pathogenic *Burkholderia* spp. would also be discovered from comparative genomic approaches. Because many *Burkholderia* species are important human or animal pathogens, information obtained from these genomic studies will be important for human health, as well as plant health.

Likewise, the genome-wide identification of single nucleotide polymorphisms between susceptible and partially resistant rice lines would greatly facilitate the identification of the genes responsible for disease resistance in rice, especially if two compared lines are near-isogenic to each other. The analysis of gene expression profiles by direct sequencing of whole transcriptomes of susceptible and resistant rice lines would also help us to understand the molecular mechanisms responsible for rice resistance to *B. glumae.*

A knowledge of the virulence mechanisms of *B. glumae* and the resistance mechanisms of rice to bacterial panicle blight will lead to novel methodologies for disease control. For example, Cho *et al*. (2007) engineered an endophytic *Burkholderia* sp. to carry an *N*-acyl homoserine lactonase gene that degrades AHL quorum-sensing signals. The engineered *Burkholderia* sp. inhibited *B. glumae* AHL molecule production and reduced the rice seedling rot symptoms caused by *B. glumae*, indicating that bacterial endophytes with the acquired ability to repress quorum sensing in *B. glumae* can be used as biological control agents (Cho *et al*., 2007). In addition, our research group recently found that the rice gene encoding a NAC4-like transcription factor was induced by *B. glumae* in a rice cultivar showing a high level of partial resistance to bacterial panicle blight (B. Shrestha and J. H. Ham, unpublished data). Transgenic rice lines overexpressing this NAC4-like transcription factor are currently being developed to determine whether they have enhanced resistance to bacterial panicle blight. A better understanding of the bacterial pathogenesis of *B. glumae* and the host resistance system for bacterial panicle blight would allow for the development of new disease control strategies and disease-resistant rice lines in more efficient ways.

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