Short communication

The roles of the shikimate pathway genes, *aroA* and *aroB*, in virulence, growth and UV tolerance of *Burkholderia glumae* strain 411gr-6

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SUMMARY

Burkholderia glumae is the major causal agent of bacterial panicle blight of rice, which is a growing disease problem for rice growers worldwide. In our previous study, some *B. glumae* strains showed pigmentation phenotypes producing at least two (yellow–green and purple) pigment compounds in casein–peptone–glucose agar medium. The *B. glumae* strains LSUPB114 and LSUPB116 are pigment-deficient mutant derivatives of the virulent and pigment-proficient strain 411gr-6, having mini-Tn5gus insertions in *aroA* encoding 3-phosphoshikimate 1-carboxyvinyltransferase and *aroB* encoding 3-dehydroquinate synthase, respectively. Both enzymes are known to be involved in the shikimate pathway, which leads to the synthesis of aromatic amino acids. Here, we demonstrate that *aroA* and *aroB* are required for normal virulence in rice and onion, growth in M9 minimal medium and tolerance to UV light, but are dispensable for the production of the phytotoxin toxoflavin. These results suggest that the shikimate pathway is involved in bacterial pathogenesis by *B. glumae* without a significant role in the production of toxoflavin, a major virulence factor of this pathogen.

Keywords: Bacterial panicle blight of rice, bacterial pigments, *Burkholderia glumae*.

Burkholderia glumae causes bacterial panicle blight (BPB) of rice, which has been a serious rice disease problem in many rice-growing countries (Ham *et al.*, 2011). A feature of pathogenic strains of *B. glumae* is the production of the bright yellow phytotoxin, toxoflavin, which is a major virulence factor (Kim *et al.*, 2004; Suzuki *et al.*, 2004). Lipase, a cell wall-degrading enzyme produced by *B. glumae*, is also considered to be an important virulence factor (Devescovi *et al.*, 2007), and has biotechnological values (Boekema *et al.*, 2007). Motility driven by flagella is important for virulence in *B. glumae*, probably because it allows the pathogen to move beyond the infection sites of the host (Kim *et al.*, 2007). The biosynthesis of toxoflavin and lipase and the biogenesis of flagella

are dependent on a quorum-sensing system mediated by *N*-octanoyl homoserine lactone (Devescovi *et al.*, 2007; Kim *et al.*, 2004, 2007). Apart from these virulence factors, the development of BPB is largely dependent on conducive weather conditions, such as prolonged high night temperatures, during the flowering and heading stages of rice (Nandakumar *et al.*, 2009; Tsushima, 1996, 2011). In addition to rice, *B. glumae* has been reported to infect and cause wilting in other field crops, including pepper, eggplant, sesame and tomato (Jeong *et al.*, 2003).

In our previous study, some strains of *B. glumae* revealed various pigmentation phenotypes producing at least two (fluorescent yellow–green and nonfluorescent purple) pigments in casamino acid–peptone–glucose (CPG) agar medium (Karki *et al.*, 2012b, 2012a). The two pigments were partially purified and the purple pigment showed an antifungal activity (Karki *et al.*, 2012b). The two-component regulatory system (TCRS), composed of the PidS sensor histidine kinase and the PidR response regulator, was shown to be essential for the production of these pigments, as well as for the elicitation of the hypersensitive response (HR) on tobacco leaves and virulence in rice panicles (Karki *et al.*, 2012a). The shikimate pathway genes *aroA* and *aroB* have also been shown to be required for the production of these pigments in the pigmenting virulent strain 411gr-6 (Karki *et al.*, 2012a) (Fig. S1, see Supporting Information). Here, we show that *aroA* and *aroB* of *B. glumae* are essential for symptom development in rice panicles and onion bulb scales, bacterial growth in M9 minimal medium and tolerance to UV light, but are dispensable for toxoflavin production.

REDUCED VIRULENCE OF *aroA* AND *aroB* MUTANTS

The bacterial strains and plasmids used in this study are listed in Table 1. The pigment-deficient mutants LSUPB114 and LSUPB116 have mini-Tn5gus insertions in *aroA*, which encodes 3-phosphoshikimate 1-carboxyvinyltransferase, and *aroB*, which encodes 3-dehydroquinate synthase, respectively (Karki *et al.*, 2012a) (Figs 1A,B and S1). For genetic complementation of the *aroB* mutant LSUPB116, a functional clone of *aroB* was constructed using the following procedures: (i) a cosmid library

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Table 1 Plasmids and bacterial strains used in this study.

Strain or plasmid	Description	Reference or source
<i>Burkholderia glumae</i>		
336gr-1	A virulent strain showing a pigment-deficient phenotype, Nit ^R	Nandakumar <i>et al.</i> (2009)
411gr-6	A virulent strain showing a pigment-proficient phenotype, Nit ^R	Nandakumar <i>et al.</i> (2009)
LSUPB114	A <i>aroA</i> ::mini-Tn5gus derivative of 411gr-6, Nit ^R , Km ^R	Karki <i>et al.</i> (2012a)
LSUPB116	A <i>aroB</i> ::mini-Tn5gus derivative of 411gr-6, Nit ^R , Km ^R	Karki <i>et al.</i> (2012a)
<i>Escherichia coli</i>		
DH5α	<i>SupE44 DlacU169 (f80 lacZDM15) hsdR17 recA1 endA1 gyrA96 thi-1 relA1</i>	Life Technologies (Carlsbad, CA, USA)
S17-1 λpir	<i>recA thi pro hsdR [res- mod+][RP4 :: 2-Tc::Mu-Km::Tn7] λ pir</i> phage lysogen	Simon <i>et al.</i> (1983)
XL1-Blue MR	Δ(<i>mcrA</i>)183 Δ(<i>mcrCB</i> - <i>hsdSMR</i> - <i>mrr</i>)173 <i>endA1 supE44 thi-1 recA1 gyrA96 relA1 lac</i>	Stratagene (La Jolla, CA, USA)
Plasmids		
pUT::miniTn5gus	A suicide vector carrying mini-Tn5gus, Ap ^R , Km ^R	de Lorenzo <i>et al.</i> (1990); Fouts <i>et al.</i> (2002)
pRK2013::Tn7	ColE1 <i>mob⁺ tra_{RK2} Δrep_{RK2} repE kan::Tn7</i> (Tp ^R , Sm ^R , Sp ^R)	Ditta <i>et al.</i> (1980)
pKNOCK _{Gm}	A suicide vector, R6K <i>ori</i> , Gm ^R	Alexeyev (1999)
pBBR1MCS-5	A broad-host-range vector, Gm ^R	Kovach <i>et al.</i> (1995)
SuperCos1	A cosmid vector, Ap ^R , Km ^R	Stratagene
pSC-A-amp/kan	A PCR cloning vector, Ap ^R , Km ^R	Stratagene
pCL317	A cosmid clone harbouring the <i>aroL/aroB</i> locus, Ap ^R	This study
pBBaroLB	A subclone of pCL317 for the 2.5-kb <i>SmaI</i> fragment harbouring both <i>aroL</i> and <i>aroB</i> in pBBR1MCS-5, Gm ^R	This study

Fig. 1 The shikimate pathway and the *aroA* and *aroB* loci. (A) Schematic description of the shikimate pathway, including the enzymes involved in each step of the pathway [adopted from Tzin & Galili (2010) and Baez-Viveros *et al.* (2007)]. The genes encoding the metabolic enzymes are indicated in parentheses. (B) Schematic diagram of *aroA* and *aroB* and their respective mutants, LSUPB114 and LSUPB116. This physical map information, including restriction enzyme sites, is based on the sequence information of *Burkholderia glumae* BGR1. The locus tag featured in the National Center for Biotechnology Information (NCBI)'s GenBank for each gene is indicated in parentheses. Black inverted triangles indicate the site of miniTn5gus insertion in each mutant. Restriction enzyme sites in the map are represented by: D, *DraIII*; B, *BamHI*; S, *SacI*; P, *PstI*; N, *NotI*; (S), *SmaI*. *SmaI* was used to subclone a 2.5-kb fragment containing *aroL* and *aroB* from the cosmid, pCL317, into the broad-host-range vector pBBR1MCS-5 (Gm^R) to create pBBaroLB.

containing the genome of *B. glumae* 336gr-1 (another highly virulent strain of *B. glumae*) was screened using an *aroB*-specific primer set, 5'-AGGTCGATACCGTGGTGCT-3' and 5'-CCTCGATCAGTCGAAGAAC-3', to identify a cosmid clone containing *aroB*; (ii) the cosmid clone, pCL317, containing *aroB* was digested with *Sma*I to obtain a 2.5-kb fragment containing *aroB* as well as *aroL* encoding a shikimate kinase; and (iii) this fragment was ligated to the *Sma*I-cut pBBR1MCS-5 (Kovach *et al.*, 1995), generating pBBaroLB (Fig. 1B).

Five single nucleotide polymorphisms (SNPs) were found in each of the *aroA* and *aroB* protein coding sequences of 411gr-6, compared with the corresponding sequences in the reference genome, *B. glumae* BGR1 (gene IDs: bglu_1g08780 for *aroA* and bglu_1g03040 for *aroB*). These SNPs caused one- and three-amino-acid differences in the predicted proteins of *aroA* and *aroB*, respectively. The nucleotide sequences of the *aroA* and *aroB* coding regions of 336gr-1, the strain from which pBBaroLB was derived, were identical to those of BGR1. The GenBank accession numbers for the *aroA* and *aroB* sequences of 411gr-1 and 336gr-1 are KJ652007 (*aroA* of 411gr-6), KJ652009 (*aroB* of 411gr-6), KJ652008 (*aroA* of 336gr-1) and KJ652010 (*aroB* of 336gr-1). The virulence of LSUPB114 (*aroA*⁻) and LSUPB116 (*aroB*⁻) was primarily examined in comparison with the parental wild-type strain, 411gr-6, based on their ability to cause symptoms on rice panicles and onion bulb scales, following the methods described previously (Karki *et al.*, 2012a). Both LSUPB114 and LSUPB116 caused substantially less symptoms on rice panicles (Fig. 2A) and onion bulb scales (Fig. 2B) compared with the wild-type, 411gr-6, indicating that both *aroA* and *aroB* play a crucial role in virulence. Virulence of LSUPB116 on onion bulb scales was restored by the complementation with pBBaroLB, indicating that the loss of virulence was a *bona fide* phenotype of the *aroB* null mutation (Fig. 2B).

Seed germination assay was also performed to verify the virulence phenotypes of the *aroA* and *aroB* mutants, as well as their growth *in planta*. The previously described method used by Devescovi *et al.* (2007) was adopted for the seed germination assay, except that the rice seeds treated with bacterial suspensions were incubated in an incubator at 30 °C without a 16-h light period per day. Briefly, *B. glumae* cells were grown on King's B agar plates at 37 °C for 18 h and resuspended in sterile water to a bacterial concentration of $\sim 1.5 \times 10^8$ colony-forming units (CFUs)/mL. Surface-sterilized rice seeds (*Oryza sativa* cv. Bengal) were incubated at 37 °C in the bacterial suspension for 72 h and transferred to a moist chamber for further incubation at 30 °C. Fifty rice seeds were incubated in each bacterial suspension and the germination rate of the treated rice seeds was determined after 6 days of incubation in the moist chamber at 30 °C. The rice seeds treated with 411gr-6 showed a germination rate of only 2.5%, whereas those treated with LSUPB114 and LSUPB116 had germination rates of 88% and 65%, respectively. LSUPB116 complemented with the *aroB* clone, pBBaroLB, regained the

germination inhibitory activity, showing a seed germination rate of 7.5% (Fig. 2C).

Bacterial growth in the germinating rice seeds during the germination assay was also examined to reveal any possible growth defect of LSUPB114 and LSUPB116 *in planta* caused by the mutation of *aroA* or *aroB*. Two rice seeds collected after 0, 2, 4 and 6 days of incubation in the moist chamber were crushed and resuspended in 10 mL of sterile water, which was then spread onto the selective medium for *B. glumae* (Kawaradani *et al.*, 2000). Bacterial colonies were counted after 24 h of incubation at 30 °C and the data were expressed as log CFU per seed (Fig. 2C). All the rice seeds used for seed germination assay and bacterial growth in germinating rice seeds were surface sterilized with 10% bleach solution before the experiments, but a residual number of bacterial cells were grown from the noninoculated control on the selective medium for *B. glumae*; therefore, the number of bacterial colonies from the noninoculated water control was subtracted from that for each bacterial treatment to determine the growth of the artificially inoculated bacteria only. The growth of *B. glumae* cells from the apparently healthy rice seeds also suggests the presence of this pathogen inside the rice seeds, which may be the primary inoculum source of BPB outbreaks in rice fields in favourable environmental conditions. The growth curve assay showed that all *B. glumae* tested, including both mutants LSUPB114 and LSUPB116, were able to multiply in rice seeds (Fig. 2C). However, the growth patterns of the mutants were somewhat different from 411gr-6 and LSUPB116(pBBaroLB); all strains showed a substantial increase in their population size (~ 2 – 2.5 times) during the first 2 days, but the two mutant strains showed reduced growth rates compared with the wild-type and the complemented *aroB* mutant during the next 4 days (Fig. 2C). Nevertheless, this result suggests that the mutation of *aroA* or *aroB* only reduces slightly the ability of *B. glumae* to multiply in rice seeds, and thus the substantially decreased germination inhibitory activities of LSUPB114 and LSUPB116 are not caused by the lack of surviving cells *in planta*. In this regard, the substantially reduced virulence of LSUPB114 and LSUPB116 in rice panicles may not be caused by a lack of multiplication of these mutants in host tissue, although bacterial multiplication was not determined in this virulence assay (Fig. 2A). This notion is also strongly supported by our previous observation from a virulence assay with rice panicles, in which other pigment-deficient derivatives of 411gr-6 could multiply in rice panicles at rates comparable with 411gr-6 in spite of their drastic reduction in virulence (Karki, 2010).

UNIMPAIRED TOXOFLAVIN PRODUCTION BY *aroA* AND *aroB* MUTANTS

Toxoflavin is known to be an important virulence factor of *B. glumae*, as mutant strains lacking the ability to produce toxoflavin are almost nonpathogenic (Kim *et al.*, 2004). To

Fig. 2 Virulence and toxoflavin production of *Burkholderia glumae* strains. (A) Representative bacterial panicle blight symptoms on rice panicles caused by the *B. glumae* strains 411gr-6, LSUPB114 and LSUPB116. The number to the right of each photograph indicates the average value of disease severity (DS) from 10 replications evaluated using a 0–9 scale at 10 days post-inoculation. In this scale, '0' indicates no symptoms present and '9' indicates that more than 90% of the panicle is symptomatic. Superscript letters indicate statistically significant differences ($P > 0.01$) among different strains. (B) Maceration symptoms on onion bulb scales caused by the *B. glumae* strains 411gr-6, LSUPB114, LSUPB116 and LSUPB116(pBBaroLB). Onion scales were inoculated with *B. glumae* strains and incubated at 30 °C for 48 h. Numerical values indicate the average macerated area (mm²) from six replications. The photograph was taken at the time of symptom evaluation. (C) Inhibition of rice seed germination by the *B. glumae* strains 411gr-6, LSUPB114, LSUPB116 and LSUPB116(pBBaroLB), and the growth patterns of these strains in the germinating rice seeds. The photograph was taken after 6 days of incubation at 30 °C. Error bars on the growth curves indicate standard deviations from three replications. CFU, colony-forming units.

determine whether the near nonpathogenic phenotypes of LSUPB114 and LSUPB116 were caused by impaired toxoflavin production, 411gr-6, LSUPB114, LSUPB116 and LSUPB116(pBBaroLB) were examined for their ability to produce toxoflavin. The extraction and quantification of toxoflavin were performed following a previously established method (Iiyama *et al.*, 1995) with some modifications. Briefly, *B. glumae* strains were grown at 37 °C overnight in 3 mL of Luria–Bertani (LB) broth, washed twice with an equal volume of fresh LB broth and resuspended in 100 µL of fresh LB broth. Then, 20 µL of each bacterial suspension was added to 15 mL of LB broth in a 250-mL flask and incubated at 37 °C for 24 h on a rotary shaker at 180 rpm. After incubation, the bacterial concentration of each culture was determined by the measurement of the optical density at 600 nm (OD₆₀₀) using a spectrophotometer (Biomate 3, Thermo Scientific, Pittsburgh, PA, USA). Toxoflavin was extracted from 1 mL of each bacterial culture by adding an equal volume of chloroform, which was then evaporated in a fume hood. The residue after complete evaporation of chloroform was dissolved in 1 mL of aqueous 80% methanol and OD₃₉₃ was measured. The OD₃₉₃ values for toxoflavin were then normalized to the OD₆₀₀ values of the corresponding bacterial samples. Interestingly, there were no significant differences in toxoflavin production among the strains tested, indicating that the production of toxoflavin was not affected by *aroA* and *aroB* (Fig. S2, see Supporting Information). The intact toxoflavin production by the *aroA* and *aroB* mutants, which exhibited near-avirulent phenotypes, strongly suggests that the shikimate pathway genes render *B. glumae* virulent through a toxoflavin-independent mechanism, and that toxoflavin production is not a trait sufficient to cause disease, even though toxoflavin is an important virulence factor of this pathogen. Similarly, Kim *et al.* (2007) observed that a *qsmR* mutant of *B. glumae* was almost avirulent in rice panicles, even though its capacity to produce toxoflavin was not impaired in the growth conditions in which the mutant strain was cultured for inoculation. Nevertheless, it cannot be ruled out that the toxoflavin production of these *aroA* and *aroB* (as well as *qsmR*) mutants might be suppressed *in planta*, but the measurement of toxoflavin present in infected rice tissue was not successful in our laboratory as a result of technical limitations.

GROWTH DEFECT OF *aroA* AND *aroB* MUTANTS IN M9 MINIMAL MEDIUM

Genes involved in the shikimate pathway are essential for the synthesis of aromatic amino acids (Herrmann and Weaver, 1999). 411gr-6, LSUPB114, LSUPB116 and LSUPB116(pBBaroLB) were grown on LB medium, M9 minimal medium (Sambrook, 2001) and M9 minimal medium supplemented with aromatic amino acids over an extended period of time, and their growth patterns were compared. For this bacterial growth assay, *B. glumae* strains were

initially grown in LB broth overnight at 37 °C, washed twice with an equal volume of fresh LB broth and resuspended in 1/10 volume of fresh LB broth. Then, 25-µL aliquots of bacterial suspensions adjusted to have the same concentration were cultured in LB broth, M9 minimal medium and M9 minimal medium amended with 0.1 g/L of L-tryptophan, L-phenylalanine and L-tyrosine, and grown at 37 °C on a rotary shaker at 180 rpm. The growth of *B. glumae* strains was observed by measuring OD₆₀₀ at 12, 16, 20, 24 and 36 h after inoculation. As shown in Fig. 3A, there was no significant difference in growth rate in LB medium between the mutants and the wild-type. In M9 medium, however, only 411gr-6 and LSUPB116(pBBaroLB) could grow (Fig. 3B). The growth defect of the *aroA* and *aroB* mutants was restored in M9 medium supplemented with the three aromatic amino acids (phenylalanine, tryptophan and tyrosine) (Fig. 3C), but not with M9 medium supplemented with only one of the three aromatic amino acids (data not shown).

GROWTH INHIBITION OF *B. GLUMAE* STRAINS BY GLYPHOSATE

Consistent with the requirement of the shikimate pathway genes *aroA* and *aroB* for growth in M9 medium, the growth of *B. glumae* 411gr-6 was suppressed by the herbicidal compound, glyphosate (Roundup®), which inhibits 5-enolpyruvylshikimate 3-phosphate synthase (AroA) (Fig. 3D) (Steinrücken and Amrhein, 1980). Growth of *B. glumae* 336gr-1, a pigment-deficient wild-type strain (Karki *et al.*, 2012b), was also inhibited by glyphosate in M9 medium, like *B. glumae* 411gr-6 (Fig. 3D), suggesting that a functional shikimate pathway is required for the growth of *B. glumae* in minimal medium.

IMPAIRED UV TOLERANCE OF *aroA* AND *aroB* MUTANTS

The wild-type strain 411gr-6, its pigment-deficient derivatives, LSUPB114 and LSUPB116, and the pigment-deficient wild-type strain, 336gr-1, were tested for their tolerance to UV light. As shown in Fig. 4, the pigment-deficient shikimate pathway mutants, LSUPB114 and LSUPB116, showed substantially reduced tolerance to UV light compared with the pigment-proficient wild-type strain, 411gr-6 (Fig. 4). Strain 411gr-6 was able to survive at an average rate of approximately 45% when exposed to UV light for 0.5 min, and was still able to survive (<5% survival rate) when exposed to UV light for 3 min (Fig. 4). In contrast, LSUPB114 and LSUPB116 were only able to survive at rates of about 12% and 7%, respectively, when exposed to UV light for 0.5 min, and were not able to survive when exposed to UV light for 3 min (Fig. 4). The pigment-deficient wild-type strain *B. glumae* 336gr-1 also showed reduced tolerance to UV light compared with *B. glumae* 411gr-6

Fig. 3 Growth of *Burkholderia glumae* strains in various medium conditions: (A) Luria–Bertani (LB); (B) M9; (C) M9 supplemented with aromatic amino acids; (D) M9 amended with glyphosate. Error bars indicate standard deviations from three replications.

Fig. 4 Effect of UV radiation on the survival of *Burkholderia glumae* strains. Error bars indicate standard deviations from three replications.

(Fig. 4). Unlike LSUPB114 and LSUPB116, *B. glumae* 336gr-1 seems to have a functional shikimate pathway because it could grow in M9 medium without the addition of the aromatic amino acids (Fig. 3D). This strongly suggests that the UV tolerance of the pigment-proficient strain 411gr-6 is primarily conferred by the pigments rather than by the shikimate pathway itself.

THE NATURE OF THE PIGMENTS

Previously, it has been shown that *B. cenocepacia* strain C5424 produces a brown melanin-like pigment, and that homogentisate (HGA) is an essential precursor for the production of this pigment (Keith *et al.*, 2007). Another recent study has reported that *B. glumae* strain AU6208 produces considerable amounts of rhamnolipids which are dark brown in colour (Costa *et al.*, 2011). Mutation of the *hppD* and *rhIC* genes of *B. glumae* (Appendix S1, see Supporting Information), which are involved in melanin biosynthesis and rhamnolipid production, respectively (Costa *et al.*, 2011; Keith *et al.*, 2007), did not produce any significant differences in pigment production (data not shown). These results

indicate that the pigments produced by 411gr-6 in CPG medium are neither melanin-like compounds nor rhamnolipids. In our previous study, two pigment compounds (one fluorescent yellow-green and the other purple) were partially purified from the pigments produced by 411gr-6, and the purple pigment showed antifungal activity (Karki *et al.*, 2012b). The structure of the purple pigment has been characterized recently as a phenazine-like compound, and that of the fluorescent yellow-green compound is still under characterization (B. S. Kim and J. H. Ham, unpublished results).

CONCLUSIONS

In this study, it was found that the shikimate pathway genes *aroA* and *aroB* are essential for the growth of *B. glumae* in M9 medium and for the virulence of this pathogen on rice panicles and onion bulb scales. These results are consistent with previous studies. A shikimate pathway mutant of *B. pseudomallei* was unable to grow in minimal medium, but growth in minimal medium was restored with the exogenous application of tryptophan, tyrosine, phenylalanine, *p*-aminobenzoic acid (PABA) and 2,3-dihydroxybenzoate (Cuccui *et al.*, 2007). Several studies have revealed that mutations of the genes involved in the shikimate pathway cause an attenuation of virulence in a wide range of bacteria (Gunel-Ozcan *et al.*, 1997; Simmons *et al.*, 1997; Stritzker *et al.*, 2004). In the plant pathogenic bacterium, *Xanthomonas oryzae* pv. *oryzae*, the shikimate pathway has been reported to be associated with pigment production and virulence (Goel *et al.*, 2001; Park *et al.*, 2009). It is noteworthy that toxoflavin, a phytotoxin of *B. glumae* which plays a key role in bacterial pathogenesis in rice, is produced normally by the shikimate pathway mutants LSUPB114 and LSUPB116. This suggests that the virulence function mediated by the shikimate pathway is independent of the action of toxoflavin. Indeed, Kim *et al.* (2007) have demonstrated that a *qsmR* mutant of *B. glumae* BGR1 is impaired in flagellar formation and nearly nonpathogenic, but still produces toxoflavin to the wild-type level in bacterial culture. This previous study and our results in this study suggest that the ability to produce toxoflavin is not always associated with the virulence of *B. glumae*. Nevertheless, we cannot rule out the possibility that the reduced virulence phenotype of the shikimate pathway mutants is a result of reduced toxoflavin production in the host, because all the assays for toxoflavin production were conducted with *B. glumae* strains grown in LB broth.

In this study, it was also revealed that the shikimate pathway-dependent pigment production contributes to the tolerance of *B. glumae* to UV light. Microbes produce a variety of pigments that are often used for bacterial taxonomy and diagnostics of bacterial diseases (Schaad *et al.*, 2001; Starr and Stephens, 1964). These pigments play various roles in the ecological fitness of microorganisms, including protection against UV radiation

(Romero-Martinez *et al.*, 2000), extreme temperatures (Paolo *et al.*, 2006), oxidants (Liu *et al.*, 2005) and antimicrobial compounds (van Duin *et al.*, 2002). Pigmentation might also play a role in the virulence of microbes by sequestering host defence responses or providing inflammatory damage to host cells (Liu and Nizet, 2009). Interestingly, pigmentation of *B. glumae* strain 411gr-6 was only observed at 30 °C, whereas toxoflavin production by this strain was maximal at 37 °C (Fig. S1) (Matsuda and Sato, 1988); however, it remains unknown how this temperature-dependent differential production of toxoflavin and pigments is co-regulated and plays a role in the parasitic fitness of *B. glumae* in nature.

ACKNOWLEDGEMENTS

This work was supported by the United States Department of Agriculture National Institute of Food and Agriculture (Hatch Project #: LAB93918 and LAB94203), the Louisiana State University Agricultural Center, the Research and Development Program of the Louisiana Board of Regents Support Fund (Grant number: LEQSF(2008-11)-RD-A-02) and the Louisiana Rice Research Board (Grant numbers: 940-38-4158 and 940-38-4173). We wish to give special thanks to Dr Jung Nam Lee for generating a cosmid library of *B. glumae* 336gr-1 and Dr Milton C. Rush for providing *B. glumae* strains. We also thank Rebecca Melanson, Bishnu Shrestha, Jingyu Peng and Surendra Osti for critical review of the manuscript.

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SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article at the publisher's web-site:

Fig. S1 Pigmentation phenotypes of *Burkholderia glumae* strains. (A) Pigment production by *B. glumae* strains on casamino acid–peptone–glucose (CPG) agar plates. Freshly grown bacterial colonies were streaked on CPG agar plates and incubated at 30 °C. (B) Pigment production by the wild-type strain 411gr-6 on CPG plates at 30 °C (left) and 37 °C (right). Photographs were taken 48 h after inoculation.

Fig. S2 Toxoflavin production by the *Burkholderia glumae* strains 411gr-6, LSUPB114, LSUPB116 and LSUPB116(pBBaroLB). Bacterial cultures were grown in Luria–Bertani (LB) broth and toxoflavin was quantified after 24 h of incubation at 37 °C in a shaking incubator at 180 rpm. Error bars indicate standard deviations from three replications. OD, optical density.

Appendix S1 Procedures for *hpdD* and *rhIC* mutation, glyphosate assay and UV tolerance assay.