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Scientific Article

Characterization and identification of *Burkholderia glumae* in seeds of rice (*Oryza sativa*) varieties planted in Mexico

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ABSTRACT

Backgrounds/Objectives. *Burkholderia glumae* is the most important pathogen transmitted through seeds that reduces the yield of rice (*Oryza sativa*). In 2022, seeds were found with symptoms of glume blotch in Mexico. The aim of this investigation was to characterize and identify the causal agent of the symptoms of glume blotch of 11 varieties of rice planted in Mexico.

Materials and Methods. A total of 37 seeds samples were analyzed. From each sample, 1 g of seed was disinfested with sodium hypochlorite and macerated in a PBS buffer and subsequently streaked onto SPG, B de King and Wilbrinks culture media. The isolated bacterial strains were characterized by API20 and identified by amplification and partial sequencing of universal and specific genes. Pathogenicity was evaluated using a 3.5 x 10^8 CFU mL^{-1} suspension on rice seeds and seedlings of the Milagro Filipino and INIFLAR-R varieties.

Results. Out of 37 samples, in nine (24.3 %), 11 isolates were taken with the morphology described for *B. glumae*. Characterization by API20 identified two groups with different metabolic profiles between the 11 strains. With PCR, the 11 strains were identified with a coverage of 100% as *B. glumae* with the universal primers 8F-1492R, and specific to *B. glumae* Bglu3F-Bglu3R and OgF-OgR. The pathogenicity of *B. glumae* was confirmed in rice seeds and seedlings of the Milagro Filipino and INIFLAR-R varieties.

Conclusion. *B. glumae* is the causal agent of the symptoms of glume blotch in rice seeds, and it was detected in seeds from seven varieties (63.6 %): GOLFO FL-16, INIFLAR-R, INIFLAR-RT, IRGA, Milagro Filipino, Morelos A-98 and PACÍFICO FL-15 of 11 rice varieties planted in Mexico.

Keywords: Bacterial panicle blight, Isolation, Varieties, Pathogenicity

INTRODUCTION

Rice (*Oryza sativa*) is one of the most important crops worldwide, since it is a staple food for more than 3.5 billion people (Faizal-Azizi and Lau, 2022). The main countries that produce this crop are Continental China, India, Indonesia, Vietnam and Thailand (FAOSTAT, 2023). In Mexico, rice is one of the four main staple crops in the diet of the country's population. Nationwide, approximately 40 thousand ha are planted, with more than 17 varieties of rice in diverse agroecological conditions, particularly in the states of Campeche, Jalisco, Morelos, Nayarit and Tamaulipas, with a production of 246 thousand t, strengthening the agro-industrial economy of the country (SIAP, 2023).

Worldwide, the rice crop is affected by different pathogens, although, *Acidovorax avenae* subsp. *avenae*, *Burkholderia glumae* and *Xanthomonas oryzae* pv. *oryzae* are among the main bacterial pathogens transmitted via seeds (Kang *et al.*, 2016). However, *B. glumae*, the causal agent of the bacterial panicle blight (BPB), stands out for its worldwide distribution and economic importance (Cui *et al.*, 2016).

B. glumae has been reported in more than 18 countries as a highly destructive pathogen in rice (Zhou, 2019) fields. In the last decade, BPB has been considered an emerging disease that has increased its incidence and severity in rice-producing countries due to climate change, leading to losses of up to 75% in the yield and quality of the grain in tropical and subtropical climates, particularly in regions with high temperatures and relative humidity (Abhishekh-Gowda *et al.*, 2022; Mizobuchi *et al.*, 2018). In the Americas, the presence of *B. glumae* has been reported in Colombia, United States, Panama, Venezuela, Nicaragua, Ecuador, Costa Rica, the Dominican Republic and Puerto Rico (CABI, 2021).

B. glumae can survive in seeds, prosper in plant tissues, colonize different microenvironments in the plant and cause severe epidemics in rice crops, due to its high transmission efficiency and dissemination via seeds (Ortega and Rojas, 2021). Consequently, the BPB disease begins with the infected seed as a primary inoculum. Subsequently, the pathogen is disseminated with the splashing of irrigation water and physical contact between healthy and diseased panicles (Ham *et al.*, 2011; Hasegawa, 2012; Zhou, 2019). On a global scale, the most frequently reported BPB symptoms are the glume blotch, the scarcity or absence of grain development, seed rot, abnormal root development and the rotting of seedlings (Sayler *et al.*, 2006), as well as blight in seedlings and panicles (Faizal-Azizi y Lau, 2022; Zhou-qi *et al.*, 2016).

Scientists of the National Forestry, Agriculture and Livestock Research Institute (INIFAP), in the Zacatepec, Morelos, experimental field are leaders in the genetic breeding of rice against diverse biotic and abiotic pathogenic agents. Since 2016, in the INIFAP field experiments in Zacatepec, Morelos, seeds of different rice varieties have been observed with glume blotches. This investigation assumes that these symptoms are caused by *Burkholderia glumae*, due to the similarity with glume blotches in rice seeds reported for this pathogen as a causal agent of the BPB disease (Ramachandran *et al.*, 2021).

The aim of this investigation was to characterize and identify the causal agent of the symptoms of glume blotch in rice varieties planted in Mexico. Identifying the causal agent of these symptoms in glumes in rice seeds is crucial to develop future prevention, management and genetic breeding strategies for rice cultivation in Mexico.

MATERIALS AND METHODS

Plant material. In 2022, 37 seed samples were analyzed with different percentages of blotch damage and necrosis in glumes in 11 varieties of rice planted in Mexico (Table 1, Figure 1), provided by the INIFAP- Zacatepec, Morelos, experimental field.

Table 1. Varieties of rice analyzed to identify the causal agent of the glume blotch in seeds.

Rice varieties	Number of samples
Azteca	4
Azteca 84	1
GOLFO FL-16	1
INIFLAR R	2
INIFLAR RT	2
IRGA	13
Milagro Filipino	7
Morelos A-98	2
Pacífico B1	1
PACÍFICO FL-15	3
Sauta	1
TOTAL 11	37

Figure 1. Symptoms of glume blotch in panicles and seeds in the rice varieties: **A.** MILAGRO FILIPINO, **B.** MORELOS A-98, **C.** PACÍFICO FL-15, **D.** IRGA. Symptoms of blotch in seeds of the rice varieties: **E.** MILAGRO FILIPINO, **F.** MORELOS A-98.

Isolation of bacteria. One gram of seed from every sample was disinfested with sodium hypochlorite (0.5% v/v) for 5 min and rinsed three times with sterile distilled water. The seeds were placed in beakers with 5 mL of PBS (Phosphate-Buffered Saline) (pH 7.2) and stirred at 125 rpm (ORBIT 1900, Labnet) for 1 h. Subsequently, they were macerated with a sterile mortar with 5 mL of PBS buffer and serial dilutions were carried out until 10^{-2}. From every dilution, 20 µL were spread using a Digralsky loop onto Petri dishes containing a sucrose-phosphate-glutamate (S-PG) semi-selective medium for *B. glumae* (Quesada-González and García-Santamaría, 2014). The dishes were incubated at 30 °C for 48 h. From the bacterial growth, individual colonies with a phenotype similar to *B. glumae* (circular, convex colonies with smooth edges and a red-purple center) (Abhishekh-Gowda *et al*., 2022) were transferred to King's B and Wilbrinks culture media (Koike, 1965) and incubated at 30 °C for 48 h. The colonies with a phenotype similar to *B. glumae* were preserved in cryogenic culture (-80 °C) in nutrient broth and glycerol (40 %) for further studies.

Physiological and biochemical characterization. The bacterial isolates were physiologically and biochemically characterized by API 20E (Biomerieux, Durhan, NC, U.S.A.), following the manufacturer's instructions. Likewise, its pectinolytic activity in potato (*Solanum tuberosum*) was evaluated, along with the hypersensitivity (RH) in tobacco (*Nicotiana tabacum* cv. *xanthi*) (Goszczynska *et al*., 2000).

Molecular identification. The genomic DNA of the bacterial isolations was obtained with the method CTAB 2% (Doyle and Doyle, 1990) from pure cultures in King's B medium with 48 h of growth at 28 °C, with modifications: During the lysis process, 300 µL of 2% CTAB a (1.4 M NaCl, 0.02 M EDTA, 0.1 M Tris-HCl pH 8, and 2 g of CTAB for every 100 mL of buffer) and 3 µL of Beta Mercaptoethanol at 10 % were added, and incubated at 55 °C for 30 min. In the precipitation, 200 µL of the aqueous phase were collected and 300 µL of isopropanol (100%) were added, at 4 °C, along with 30 µL of ammonium acetate (7.5 M), and gently mixed by inversion. The concentration and purity of the DNA samples were analyzed by UV light spectrophotometry at 260 nm with a NanoDrop™ 2000 (Thermo Fisher Scientific). The integrity was evaluated by electrophoresis in agarose gel (Invitrogen™) at 1% at 70V for 90 min. Molecular identification was performed by endpoint PCR with 100 ng μL^{-1} of DNA from each bacterial isolation using universal (8F-1492R) and specific (Bglu3F-BgluR, OgF-OgR) primers for *B. glumae* (Table 2). The PCR GOTAQ® Flexi DNA Polymerase (Ref. M8295, Promega) kit was used in a C1000 Touch TM Thermal Cycler (Eppendorf).

The PCR conditions for the 8F-1492R primers were an initial denaturalization at 94 °C for 5 min, followed by 35 cycles at 94 °C for 60 s, 57 °C for 45 s, 72 °C for 60 s and a final extension at 72 °C for 8 min. For the specific primers Bglu3F-Bglu3R, the PCR conditions were an initial denaturalization at 95 °C for 2 min, followed by 35 cycles at 95 °C for 30 s, 54 °C for 30 s, 72 °C for 30 s and a final extension at 72 °C for 7 min. For the primers OgF-OgR, an initial denaturalization at 95 °C for 2 min, followed by 35 cycles at 95 °C for 20 s, 63 °C for 30 s, 72 °C for 30 s and an extension at 72 °C for 7 min.

Table 2. Primers used for the identification of bacteria isolated from seeds with symptoms of glume blotch in rice varieties planted in Mexico.

Primer	Sequence (5´-3´)	Target gene	Predicted size of PCR product (bp)	Reference	Annealing temperature
8F	AGAGTTTGATCCTGGCTCAG	16S rRNA	1500	Galkiewicz y Kellogg, 2008	57°C
1492R	GGTTACCTTGTTACGACTT				
Bglu3F	GTCACGATCGCTCTGTTGTT	Putative protein	174	Lee *et al*., 2018	54°C
Bglu3R	CATGAATCCACGAAGCCGAG				
Og-F	CACCTGGGTAGTCTCTGTAGG	16S-23S rRNA Intergenic spacer region	402	Kang *et al*., 2016	63°C
Og-R	ACGAGTCTGTCTCGCTCT				

The amplified products were detected in a tris-ethylene-diaminetetraacetic acid (EDTA) agarose gel at al 1.5%, purified and sequenced bidirectionally with the three pairs of primers in the company Macrogen Inc. (Seoul, South Korea). The submitted sequences were analyzed and edited with the program BioEdit to generate the consensus sequences of each strain. The consensus sequences were compared with the nucleotide sequences in the National Center for Biotechnology Information (NCBI; https://www.ncbi.nlm.nih.gov/) database using the BLASTn 2.2 (Basic Local Alignment Search Tool) program. The polymorphism of the strains in the 16S rRNA was determined with the multiple alignment of the sequences from Mexico and those available in the NCBI with the program CLUSTAL W, implemented in the program MEGA 11 (Tamura *et al.*, 2021). Based on the 16S rRNA sequences of the 11 strains, a phylogenetic tree was generated and analyzed with the Maximum Likelihood (ML) method 500 Bootstrap replications. The trees were generated using the sequence of a species of the *Burkholderia* genus as outgroup.

Pathogenicity in rice seed. A pure culture of the *B. glumae* CPM01 strain was used, identified in this study, with 48 h of growth in King's B. The pathogenicity tests were carried out in Milagro Filipino variety seeds without any symptoms of glume blotch. The inoculation was carried out following the protocols by Li *et al*. (2016) and Ura *et al*. (2006) with some modifications. Fifteen seeds were disinfested with 70% ethanol (v/v), followed by 0.5% sodium hypochlorite (v/v) for 5 min and three washes with sterile distilled water. Subsequently, the seeds were inoculated by submersion in a bacterial suspension with 3.5 x 10^8 CFU mL^{-1} for 1 h and placed in germination boxes (BOX LARGE, THOMAS SCIENTIFIC, USA). The boxes were kept under laboratory conditions at a constant temperature of 30 °C and a relative humidity of >70%. Fifteen

days after inoculation (dai), symptoms were found on the seed, root and stem of seedlings. The assay was carried out in a completely randomized design with three repetitions per treatment. Treatment 1 consisted of seeds inoculated with *B. glumae* strain CPM01 and the treatment of 2 non-inoculated seeds (submersion of seeds in sterile distilled water) (control).

Pathogenicity in rice seedlings. The pathogenicity tests were performed in healthy seedlings of the Milagro Filipino and INIFLAR-R varieties, which are extensively planted in Mexico (SIAP, 2023). The seedlings were obtained from healthy and disinfested seeds as described earlier. The seeds were planted in Styrofoam cups (1 kg) with sterile soil (NUTRIGARDEN). Inoculation was performed when the seedlings developed their fourth leaf (Flórez-Zapata and Uribe-Vélez, 2011). Using a sterilized syringe, a lesion was made on the stem of the seedling and 3 mL of a bacterial suspension were inoculated by spraying, along with 3.5×10^8 CFU mL^{-1} of *B. glumae* (strain CPM01). The seedlings were maintained under greenhouse conditions at 28-30 °C, with a relative humidity >70%. Fifteen days after inoculation, symptoms were observed on the stem. The assay was carried out in a completely randomized design with three repetitions per treatment. Treatment 1 consisted of Milagro Filipino seedlings inoculated with *B. glumae* strain CPM01. Treatment 2 consisted of INIFLAR-R seedlings inoculated with *B. glumae* strain CPM01; treatments 3 and 4, of non-inoculated Milagro Filipino and INIFLAR-R seedlings (with lesion on the stem and sprayed with 3 mL of sterile distilled water) (control). Out of the tissues (seed and stem) in which the symptoms developed, the inoculated bacteria was reisolated in King's B culture medium and identified by PCR with the specific primers (Bglu3 and Og) for *B. glumae*.

RESULTS

Isolation of *B. glumae*. In nine (24.3%) of the 37 rice seed samples analyzed, 11 bacterial colonies were isolated. In the sucarose-phosphate-glutamate (S-PG), the colonies displayed slow growth, a convex circular shape, with smooth edges and a red-purple center. These phenotypic characteristics are correlated with what has been reported in this culture medium for *B. glumae* colonies (Ura *et al*., 2006; Quesada-González and García-Santamaría, 2014). In the King's B and Wilbrinks culture media, the bacteria formed yellow colonies, raised circular colonies, mucoid and with smooth edges (Figure 2). In the three media, a yellow diffusible pigmentation was observed around the bacterial colonies.

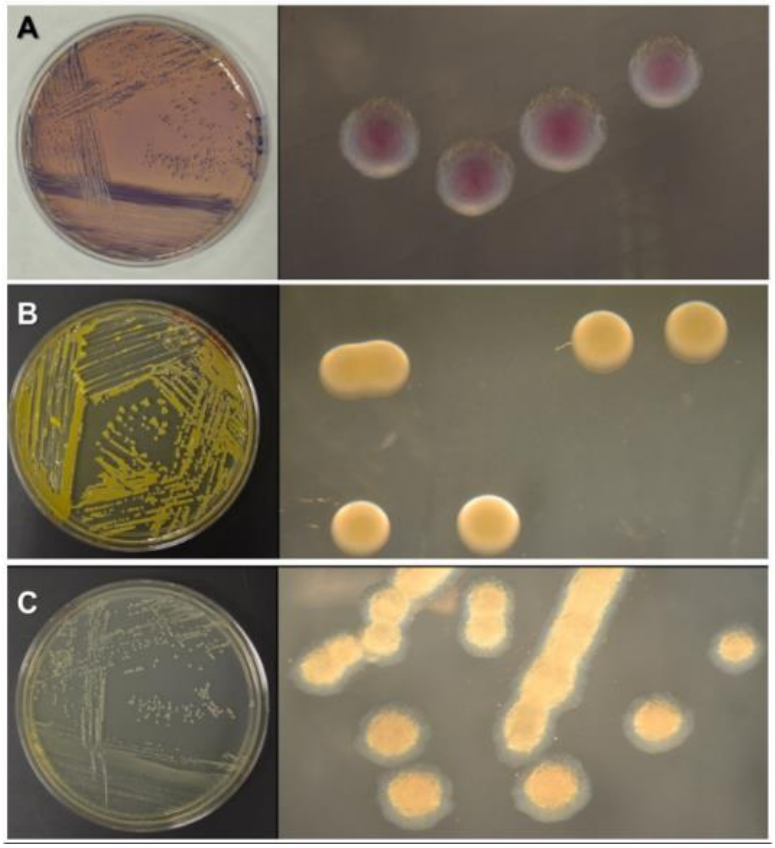

Figure 2. Morphological characteristics of the bacterial colonies isolated from rice seeds with symptoms of glume blotch. Culture of the colonies at 28 °C for 72 h in a culture medium. **A.** Sucrose-phosphate-glutamate (S-PG), **B.** King's B, **C.** Wilbrinks.

Out of the 11 rice varieties analyzed, the bacterial strains with phenotypes similar to *B. glumae* were isolated from seven varieties (63.6%): GOLFO FL-16, INIFLAR R, INIFLAR RT, IRGA, Milagro Filipino, Morelos A-98 and PACÍFICO FL-15. No strains were isolated with this phenotype from four varieties: Azteca, Azteca 84, Pacífico B1 and Sauta (Table 3).

Table 3. Bacterial strains with a *B. glumae* phenotype isolated with symptoms of glume blotch in rice varieties planted in Mexico.

	Variety	No. of isolates	Strain ID
	Azteca	0	-
	Azteca 84	0	-
	GOLFO FL-16	1	CPM04
	INIFLAR-R	1	CPM01
	INIFLAR-RT	2	CPM02, CPM03
	IRGA	1	CPJ01
	Milagro Filipino	3	CPN01, CPN03, CPJ02
	Morelos A-98	1	CPN02
	PACÍFICO B1	0	-
	PACÍFICO FL-15	2	CPM05, CPM06
	Sauta	0	-
TOTAL		11	

Physiological and biochemical characterization. The 11 strains were Gram-negative, they caused soft rot in potato and induced RH in tobacco. The results of the characterization by API 20E differentiated two groups based on their metabolic profiles. Group 1 differentiated strains (n= 8) isolated from the varieties GOLFO FL-16 (CPM04), INIFLAR R (CPM01), INIFLAR-RT (CPM02 and CPM03), Milagro Filipino (CPN01), Morelos A-98 (CPN02) and PACÍFICO FL-15 (CPM05 and CPM06). Group 2 differentiated the strains (n= 3) isolated from the varieteis IRGA (CPJ01) and Milagro Filipino (CPN01 and CPJ02) (Tables 3 and 4).

Table 4. Physiological and biochemical characterización with API 20E from 11 strains with a *B. glumae* phenotype of rice seeds with symptoms of necrosis in glumes.

Test	Reaction	Strains Group1[y] (n= 8)	Strains Group2[z] (n=3)
ONPG	β-Galactosidase	+	+
ADH	Arginine dihydrolase	-	+
LDC	Lysine decarboxilase	-	+
ODC	Ornithine descarboxilase	-	+
CIT	Citrate utilization	-	+
H2S	H_2S production	-	-
URE	Urea hydrolysis	-	+
GEL	Gelatin hydrolysis	+	+
GLU	Glucose fermentation	-	-
MAN	Mannitol	+	-
INO	Inositol	+	+
SOR	Sorbitol	+	-
RHA	Rhamnose	-	-
SAC	Sucrose	-	-
MEL	Melibiose	+	+
AMY	Amigdalin	+	-
ARA	Arabinose	+	+

[y]Group 1: Rice varieties and *B. glumae* strains (n=8) GOLFO FL-16 (CPM04), INIFLAR-R (CPM01), INIFLAR- RT (CPM02 and CPM03), Milagro Filipino (CPN01), Morelos A-98 (CPN02) and PACÍFICO FL-15 (CPM05 and CPM06).
[z]Group 2: Rice varieties and *B. glumae* strains (n=3) de *B. glumae*: IRGA (CPJ01) and Milagro Filipino (CPN03 and CPJ02).
Differences in the metabolic profile between the two groups aere found with ADH, LDC, ODC, CIT, URE, MAN, SOR y AMY.

Molecular identification of *B. glumae*. From the DNA of the 11 isolated bacterial strains found, fragments of 1500, 402 and 174 bp were amplified with the 8F-1492R universal primers, and the specific primers for *B. glumae* Bglu3F-Bglu3R and OgF-OgR, respectively (Figure 3).

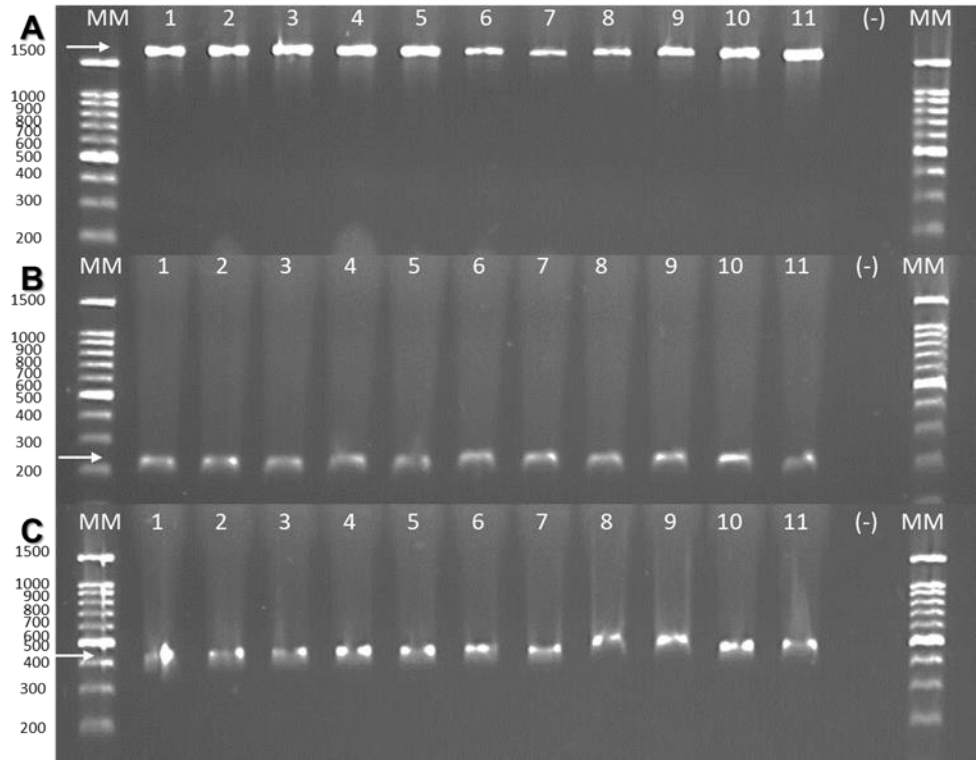

Figure 3. Detection of *B. glumae* by endpoint PCR. Partial amplification of the following genes: **A.** 16S rRNA with primers 8F-1492R (1500 bp). **B.** Putative protein with primers Bglu3 (174 bp). **C.** 16S-23S rRNA intergenic spacer region with primers Og (402 bp). Lane 1: INIFLAR-R (CPM01); lanes 2-3: INIFLAR-RT (CPM02 and CPM03, respectively); Lane 4: GOLFO FL-16 (CPM04); lanes 5-6: PACÍFICO FL-15 (CPM05 and CPM06, respectively); Lanes 7-9: Milagro Filipino (CPN01, CPN03 and CPJ02, respectively); Lane 10: IRGA (CPJ01) and lane 11: Morelos A-98 (CPN02). MM: Molecular marker 100 bp (GOLDBIO); (-): Negative control (sterile distilled water).

The bioinformatic analysis of the 16S rRNA gene fragments, putative protein and the 16S-23S rRNA intergenic spacer region of the 11 strains indicated an identity value between 99 and 100% with *B. glumae* sequence CPO23203.1. reported in the NCBI GenBank (Table 5, Figure 4), which corresponds to the complete sequence of the genome of *B. glumae* strain 336gr-1, isolated from *O. sativa* in Louisiana, United States.

Table 5. Molecular identification of 11 strains isolated from seeds of rice varieties planted in Mexico with symptoms of glume blotch.

Target gene	Strains	Query coverage	Percent Identity	Predicted size of PCR (pb)	Scientific Name	NCBI Accesion no.
16S rRNA	11	100	99-100	1500	*B. glumae*	CP023203.1
Putative protein	11	100	99-100	174	*B. glumae*	CP023203.1
16S-23S rRNA Intergenic spacer region	11	100	99-100	402	*B. glumae*	CP023203.1

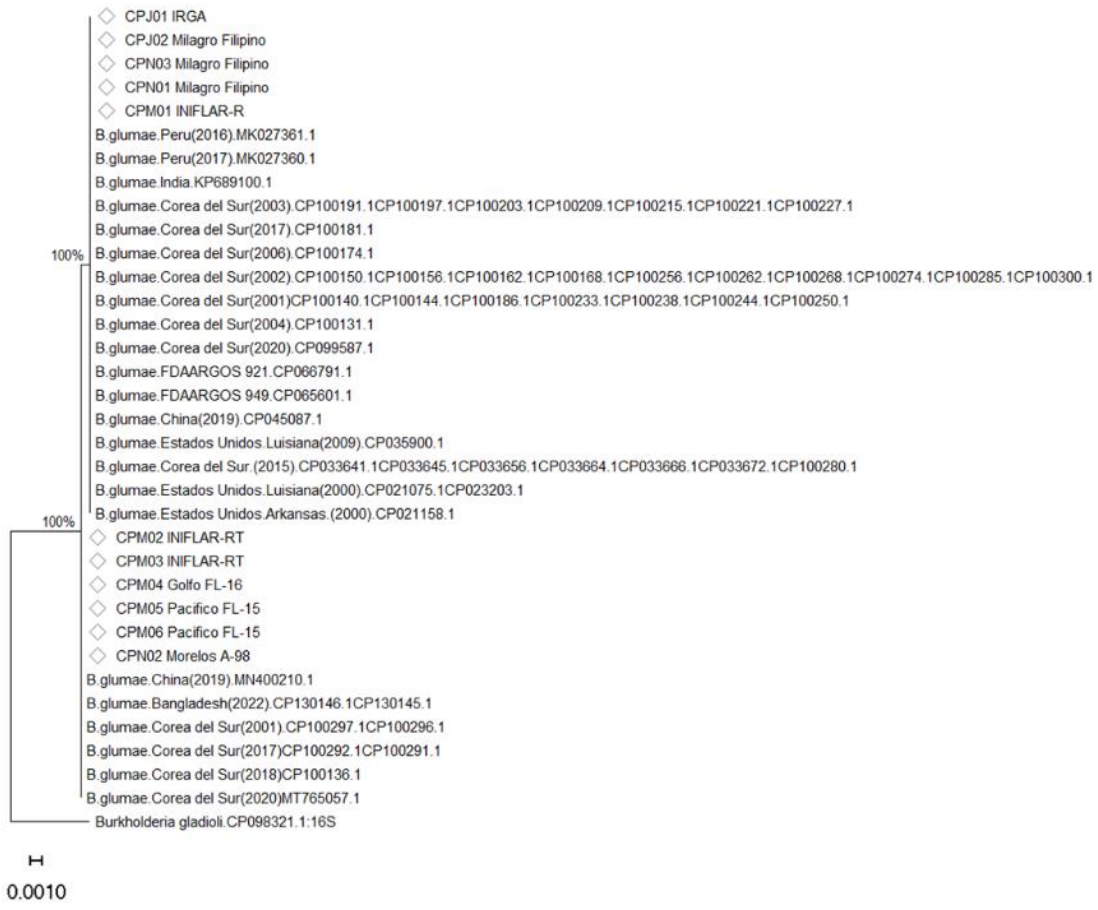

Figure 4. Phylogenetic analysis of the fragment of region 16S rRNA of 11 strains of *B. glumae* isolated in Mexico. Consensus phylogenetic tree inferred by the Maximum likelihood method. Model GTR+G+I and 500 Bootstrap replicates. The *Burkholderia gladioli* sequence CP098321.1 was included as an outgroup. ◊= *B. glumae* strains isolated from seeds with symptoms of glume blotch from rice varieties planted in Mexico.

The 16S rRNA nucleotide sequences of the 11 *B. glumae* strains were registered in the NCBI GenBank with accession numbers CPM01 (PV435843), CPM02 (PV435844), CPM03 (PV435845), CPM04 (PV435846), CPM05 (PV435847), CPM06 (PV435848), CPN01 (PV435849), CPN02 (PV435850), CPN03 (PV435851), CPJ01 (PV435852) and CPJ02 (PV435853).

Pathogenicity of *B. glumae* in rice seeds and seedlings. At 15 ddi, *B. glumae* CPM01 caused necrosis and rot in 100% of seeds, roots and stem of Milagro Filipino variety seedlings. Likewise, the bacteria inoculated in the Milagro Filipino and INIFLAR-R seedling stems caused necrosis and stem rot, as well as the abnormal development of roots and a reduction in seedling growth. No symptoms were observed in the seeds and seedlings of the control treatment (Figure 5).

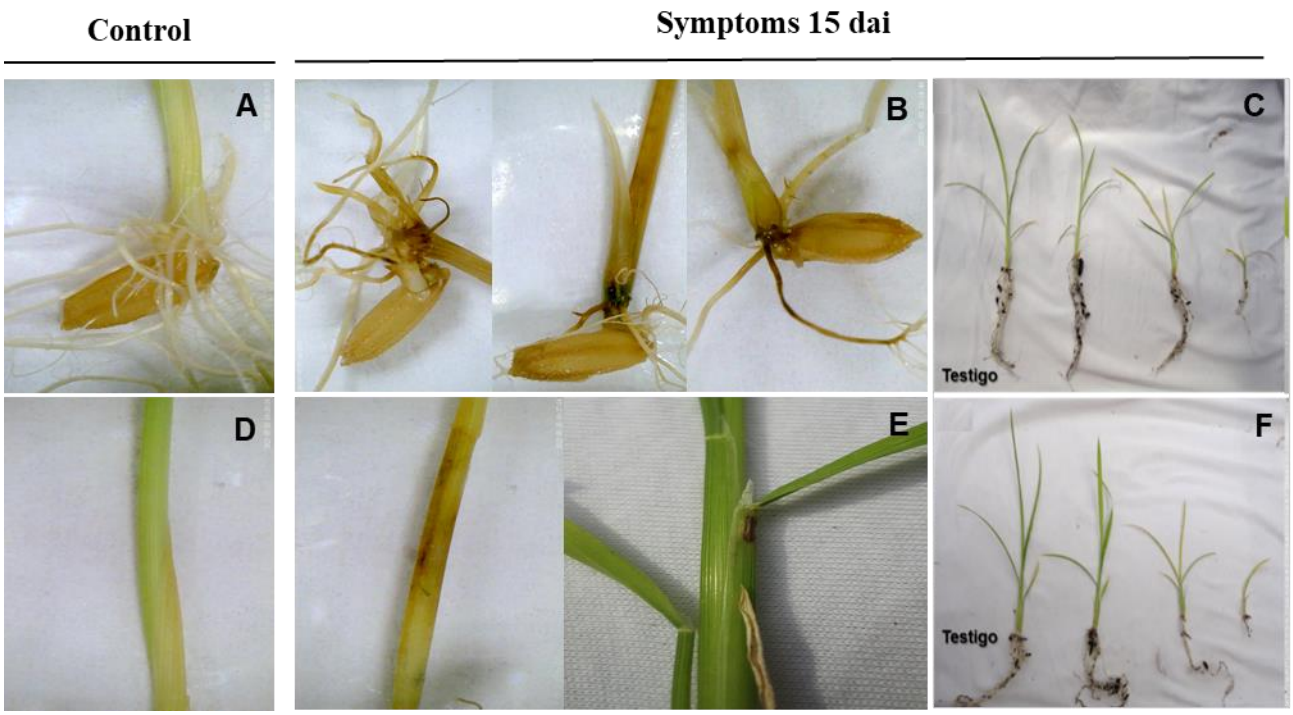

Figure 5. Symptoms induced by *B. glumae* CPM01 15 dai in rice seeds and seedlings. MILAGRO FILIPINO variety: **A.** Control, submersion of seed in sterile distilled water. **B.** Necrosis and rotting of seeds, roots and stem. **C.** Reduction in growth and abnormal root development. INIFLAR-R variety: **D.** Control, spraying of sterile distilled water on stem. **E.** Necrosis and rotting of seedling stems. **F.** Reduction in growth and abnormal development of roots.

The bacteria inoculated in seeds and seedlings were reisolated and identified by PCR as *B. glumae* with specific primers Bglu3 and Og described earlier, confirming the pathogenicity of the bacteria.

DISCUSSION

Rice is a staple crop for over 50% of the world's population. In this investigation, *B. glumae* was isolated and identified in rice seeds with symptoms of glume blotch. Out of 37 seed samples analyzed, bacterial colonies were isolated in nine of them (24.3%). The bacteria developed round, convex, smooth and yellow colonies with red-purple centers in the semiselective medium S-PG, which coincides with the morphology described for *B. glumae* in other investigations (Quesada-González and García-Santamaría, 2014; Ramachandran *et al.,* 2021; Tsushima *et al.,* 1986). Likewise, all colonies produced a diffusible yellow pigment in the King's B and Wilbrinks culture media. This pigment, known as toxoflavin, is a toxin that is considered a significant virulence factor for the pathogenicity of *B. glumae* in rice plants (Gil *et al.,* 2022; Maurya *et al.,* 2023).

The identification of *B. glumae* was confirmed by PCR with universal and specific primers. Based on the partial sequences of the 16S rRNA genes, putative protein and 16S-23S rRNA intergenic spacer region, the 11 isolated strains displayed a coverage of 100% and identity with the *B. glumae* strain CP023203.1 deposited in the NCBI. The analysis

of specific sequences for these bacteria, along with the putative protein gene (Bglu3), enables the precise identification of *B. glumae* and its discrimination among *B. gladioli*, which present a phenotypical similarity with the *B. glumae* colonies, as well as in the mechanism of biosynthesis of toxoflavin, causing identical symptoms to those of *B. glumae* in rice seeds and panicles (Lee *et al.*, 2018; Nandakumar *et al.,* 2009). Likewise, the analysis of the sequence of the 16S-23S rRNA intergenic spacer region (Og) helps separate *B. glumae* from other pathogens transmitted by rice seeds, such as *Xanthomonas oryzae* pv. *oryzae* and *Acidovorax avenae* subsp. *avenae* (Kang *et al.*, 2016). *Burkholderia glumae* is a pathogen that is transmitted through seeds (Cui *et al.*, 2016; Chompa *et al.,* 2022) and it has been proven that the infection of rice seeds is the main source of dissemination of the bacteria within a country (Abhishekh-Gowda *et al.,* 2022). The presence of *B. glumae* is estimated to cause losses of up to 75% in grain yield and quality, depending on the rice variety in regions with high temperatures and relative humidity (Fory *et al.*, 2014; Shew *et al.,* 2019), as in the case of the regions in Mexico where rice is planted.

In this investigation, *B. glumae* was isolated from seeds from seven (63.6%) rice varieties: GOLFO FL-16, INIFLAR-R, INIFLAR-RT, IRGA, Milagro Filipino, Morelos A-98 and PACÍFICO FL-15. The bacteria was isolated with a higher frequency (43%) from the seeds of the variety Milagro Filipino, which is highly relevant, since this variety currently covers 86.8% of the surface planted in Mexico and is cultivated in eight of the 12 rice-producing states, followed by the Morelos variety, with 7.6% of the surface planted nationwide (SIAP, 2023).

So far, neither the chemical and biological control methods nor cultural practices have been efficient for the optimum management of BPB (Mizobuchi *et al.,* 2018; Ortega and Rojas, 2021). The genetic resistance of plants is considered one of the main strategies for the control of bacterial diseases; however, on a global scale, no rice varieties or hybrids have been found that are completely resistant to *B. glumae*. Several varieties have been evaluated in order to identify materials with some degree of tolerance or resistance to the bacteria, although the varieties planted have shown high levels of susceptibility and moderate levels of resistance (Mizobuchi *et al*., 2018; Shew *et al*., 2019). Nevertheless, resistance to *B. glumae* in rice has been reported to be quantitative (associated to multiple genes), and therefore the mapping of Quantitative Trait Locus (QTL) has been suggested in rice varieties with these levels of resistance (Pilet-Nayel *et al*., 2017).

The inoculation of *B. glumae* strain CPM01 identified in this study confirmed the pathogenicity of the bacteria in rice seeds and seedlings of the Milagro Filipino and INIFLAR- R varieties, both of which have been planted extensively in Mexico. A greater severity was observed in seeds, roots and stems of the Milagro Filipino variety, which suggests that there could be different responses of susceptibility to the infection of *B. glumae* in seedlings of the rice varieties planted in Mexico.

To date, there are no reports of *B. glumae* causing the BPB disease in rice in Mexico. However, this study shows the presence of *B. glumae* in seeds with symptoms of glume blotch in seven of the 11 rice varieties planted in Mexico, including the variety Milagro Filipino, which covers 86.8% of the national surface planted (SIAP, 2023). Due to this, it is necessary to further investigate the origin and route of entry into the country, as well as the genetic variability and virulence of the *B. glumae* populations identified in this study. In countries with high rice production rates, such as Thailand, Malaysia and India,

knowledge on the genetic variability and virulence of *B. glumae* has been important to develop strategies for the management of BPB (Jungkhun *et al.*, 2021; Kumar *et al.*, 2023; Ramachandran *et al.*, 2021; Sreenayana *et al.*, 2024).

In 2016, rice genetic materials were evaluated under conditions of the dry and humid Mexican tropics. From this evaluation, the varieties INIFLAR- RT, INIFLAR-R, Pacífico FL-15 and Golfo FL-16 were selected, as they displayed a high potential for yield, a high industrial quality of the grain and a moderate resistance to *Magnaporthe oryzae* (synonymous with *Pyricularia oryzae*), to the rice hoja blanca virus (RHBV) and to *Bipolaris oryzae*, causal agents of the "rice blast", "hoja blanca" y "brown spot" diseases, respectively (INIFAP-Fondo Sectorial SAGARPA-CONACyT, 2016). Nevertheless, this evaluation did not consider *B. glumae*.

The incorporation of resistance to *B. glumae* in the rice varieties requires a careful evaluation of the response of the available genetic materials against the pathogen, in order to identify those with the highest resistance (Zhou, 2019). Due to the global relevance of *B. glumae* in rice, in Mexico it is crucial to evaluate the level of resistance against *B. glumae* of the rice varieties currently planted, since the degree of susceptibility to this pathogen under the environmental conditions that prevail in the areas in which rice is grown is unknown. As a consequence, the results of this investigation provide relevant information for the production of rice in Mexico, since the early and accurate detection of *B. glumae* in seeds is considered a crucial prerequisite for the development of epidemiological studies, as well as to limit its dissemination and to implement strategies to contain, prevent and for the genetic breeding of rice varieties with resistance to this pathogen (Abhishekh-Gowda *et al.*, 2022; Faizal-Azzizi and Lau, 2022; Kumar *et al.*, 2023).

CONCLUSIONS

Burkholderia glumae is the causal agent of glume blotch in rice seeds and was found in seven of the eleven rice varieties planted in Mexico (GOLFO FL-16, INIFLAR-R, INIFLAR-RT, IRGA, Milagro Filipino, Morelos A-98 and PACÍFICO FL-15). The bacterium was more prevalent in the variety Milagro Filipino, which is one of the most extensively planted varieties in different regions of the country. This investigation is the first to report the presence of *B. glumae* in Mexico in rice seeds with symptoms of glume blotch. These results are of great importance for the development of future investigations centered on the establishment of containment and management strategies, as well as on the genetic breeding of rice varieties planted in Mexico with resistance to *B. glumae*.

Conflict of interest

The authors declare there is no conflict of interest related with this investigation.

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Contributions of the author

Gabriel Alejandro Hernández-Nava: Performed the experiments, gathered data and wrote his PhD dissertation based on this investigation. **Sergio Aranda-Ocampo**: Conception, design and conduction of research, writing, revision and editing of the original draft. **Guadalupe Valdovinos-Ponce, Obdulia Segura-León, Mónica Osnaya-González, Eridani García-Vázquez, Sergio Ramírez-Rojas and Leonardo Hernández-Aragón**: Research, methodology, revision of the original draft. All authors read and approved the final manuscript.

REFERENCES

Abhishekh-Gowda HR, Tripathi R, Tewari R and Vishunavat K. 2022. Morphological and molecular characterization of *Burkholderia glumae* causing panicle blight of paddy. Physiological and Molecular Plant Pathology, 117:101755. https://doi.org/10.1016/j.pmpp.2021.101755

CABI. 2021. *Burkholderia glumae* (bacterial grain rot). Consultado en internet el 03 de mayo de 2023. https://doi.org/10.1079/cabicompendium.44964

Chompa SS, Akter A, Sadeq ABM, Rahman ME, Rashid HO, Ibnat N and Hossain MB. 2022. An Overview of Major Bacterial Diseases of Rice and Management Strategies for Their Control in Malaysia. Global Scientific Journal, 10(4): 1074-1102.

Cui Z, Zhu B, Xie G, Li B and Huang S. W. 2016. Research status and prospect of *Burkholderia glumae*, the pathogen causing bacterial panicle blight. Rice Science 22: 111-118.

Doyle JJ and Doyle JL. 1990. Isolation of plant DNA from fresh tissue. Focus, 12:13-15. https://doi.org/10.1016/j.rsci.2016.01.007

Faizal-Azizi MM and Lau HY. 2022. Advanced diagnostic approaches developed for the global menace of rice diseases: a Review. Canadian Journal of Plant Pathology, 44(5):627-651. https://doi.org/10.1080/07060661.2022.2053588

FAOSTAT. 2023. Food Agriculture Organization Statistics. URL https://www.fao.org/faostat/es/#data/QCL [1 September 2023].

Flórez-Zapata NMV and Uribe-Vélez D. 2011. Determination of the infection of *Burkholderia glumae* in commercial Colombian rice varieties. Revista - Facultad Nacional de Agronomía Medellín, 64(2): 6093-6104.

Fory PA, Triplett L, Ballen C, Abello JF, Duitama J, Aricapa MG, Prado GA, Correa F, Hamilton J, Leach, JE, Tohme J and Mosquera GM. 2014. Comparative analysis of two emerging rice seed bacterial pathogens. Phytopathology, 104:436-444. https://doi.org/10.1094/PHYTO-07-13-0186-RPMid:24261408

Galkiewicz JP and Kellogg CA. 2008. Cross-Kingdom amplification using bacteria-specific primers: complications for studies of coral microbial ecology. Applied Environmental Microbiology, 74(24): 7828-7831. http://doi.org/10.1128/AEM.01303-08

Gil J, Ortega L, Rojas JA and Rojas CM. 2022. Genome Sequence Resource of *Burkholderia glumae* UAPB13. PhytoFrontiers, 2(2):140-142. https://doi.org/10.1094/PHYTOFR-10-21-0070-A

Goszczynska T, Serfontein JJ and Serfontein S. 2000. Introduction to practical phytobacteriology: a manual for phytobacteriology by SAFRINET, SDC Switzerland. 83 p. URL https://www.researchgate.net/publication/237021880_Introduction_to_Practical_Phytobacteriology_A_manual_for_phytobacteriology. [1 May 2023].

Ham JH, Melanson, RA and Rush, MC. 2011 *Burkholderia glumae*: next major pathogen of rice? Molecular Plant Pathology, 12:329-339. https://doi.org/10.1111/j.1364-3703.2010.00676.x

Hasegawa M. 2012 Chemical control of rice plant rot after transplanting caused by *Burklholderia glumae*. Japan Journal Phytopathology, 78:553.

Jungkhun N, Gomes de Farias AR, Watcharachaiyakup J, Kositcharoenkul N, Ham JH and Patarapuwadol, S. 2021. Phylogenetic characterization and genome sequence analysis of *Burkholderia glumae* strains isolated in Thailand as the causal agent of rice bacterial panicle blight. Pathogens, 11(6): 676. https://doi.org/10.3390/pathogens11060676

Kang IJ, Kang MH, Noh TH, Shim HK, Shin DB and Heu S. 2016. Simultaneous Detection of Three Bacterial Seed-Borne Diseases in Rice Using Multiplex Polymerase Chain Reaction. Plant Pathology Journal, 32(6): 575-579. 10.5423/PPJ.NT.05.2016.0118.

Koike H. 1965. The aluminum-cap method for testing sugarcane varieties against leaf scald disease. Phytopathology, 55: 317-319.

Kumar S, Mondal KK, Ghoshal T, Kulshreshtha A, Sreenayana B, Lakshmi MA, Mrutyunjaya S, Rashmi ER, Kalaivanan NS, Kundu A and Mani C. 2023. Phylogenetic analysis, molecular characterization and virulence profiling based on toxoflavin gene of an Indian BG1 strain of *Burkholderia glumae* causing panicle blight of rice. 3 Biotech, 13(7):239. https://doi.org/10.1007/s13205-023-03660-6

Lee C, Lee HH, Mannaa M, Kim N, Park J, Kim J and Seo YS. 2018. Genomics-based Sensitive and Specific Novel Primers for Simultaneous Detection of *Burkholderia glumae* and *Burkholderia gladioli* in Rice Seeds. The Plant Pathology Journal 34(6): 490-498. https://doi.org/10.5423/PPJ.OA.07.2018.0136

Li L, Wang L, Liu LM, Hou YX, Li QQ and Huang SW. 2016. Infection Process of *Burkholderia glumae* Before Booting Stage of Rice. Journal Phytopathological, 164:825-832. 10.1111/jph.12502.

Maurya R, Singh M, Srivastava D and Rathor S. 2023. Insights from the Conventional Breeding and Molecular Approaches for Rice Bacterial Panicle Blight Disease Resistance. In Bacterial Diseases of Rice and Their Management. Apple Academic Press. pp. 129-142. https://doi.org/10.1201/9781003331629-7

Mizobuchi R, Fukuok, S, Tsuiki C, Tsushima S and Sato H. 2018. Evaluation of major rice cultivars for resistance to bacterial seedling rot caused by *Burkholderia glumae* and identification of Japanese standard cultivars for resistance assessments. Breeding Science, 70(2):221-230. https://doi.org/10.1270/jsbbs.19117

Nandakumar R, Shahjahan AKM, Yuan XL, Dickstein ER, Groth DE, Clark CA, Cartwright RD and Rush MC. 2009. *Burkholderia glumae* and *B. gladioli* cause bacterial panicle blight in rice in the southern United States. Plant Disease, 93(9):896-905. https://doi.org/10.1094/PDIS-93-9-0896

Ortega L and Rojas CM. 2021. Bacterial Panicle Blight and *Burkholderia glumae*: From pathogen biology to disease control. Phytopathology, 111(5):772-778. https://doi.org/10.1094/PHYTO-09-20-0401-RVW

Pilet-Nayel ML, Moury B, Caffier V, Montarry J, Kerlan MC, Fournet S, Durel CE and Delourme R. 2017. Quantitative resistance to plant pathogens in pyramiding strategies for durable crop protection. Frontiers in Plant Science 8:1838. DOI: https://doi.org/10.3389/fpls.2017.01838

Quesada-González A and García-Santamaría F. 2014. *Burkholderia glumae* in the rice crop in Costa Rica. Agronomía Mesoamericana. 25(2): 371-381. https://doi.org/10.15517/am.v25i2.15452

Ramachandran K, Vijaya SI and Ahmad FN. 2021 Characterization and identification of *Burkholderia glumae* as the causal pathogen of bacterial panicle blight of rice (*Oryza sativa* L.) in Malaysian rice granaries. Journal of General Plant Pathology, 87: 164–169. https://doi.org/10.1007/s10327-021-00991-1

Sayler RJ, Cartwright RD and Yang YN. 2006. Genetic characterization and real-time PCR detection of *Burkholderia glumae*, a newly emerging bacterial pathogen of rice in the United States. Plant Disease, 90 (5):603-610. https://doi.org/10.1094/PD-90-0603

Shew AM, Durand-Morat A, Nalley LL, Zhou XG, Rojas C and Thoma G. 2019. Warming increases bacterial panicle blight (*Burkholderia glumae*) occurrences and impacts on USA rice production. PLoS ONE, 14: e0219199. https://doi.org/10.1371/journal.pone.0219199

SIAP. 2023. Servicio de Información Agroalimentaria y Pesquera. Anuario Estadístico de la Producción Agrícola. URL https://nube.siap.gob.mx/cierreagricola/ [1 August 2023].

Sreenayana B, Mondal KK, Mathiyalagan N, Shanmugam NK, Kumar S, Reddy SM and Manil C. 2024. Molecular characterization and evaluation of novel management options for *Burkholderia glumae* BG1, the causative agent of panicle blight of rice (*Oryza sativa* L.). Molecular Biology Report, 51:519. https://doi.org/10.1007/s11033-024-09498-2

Tsushima S, Wakimoto S and Mogi S. 1986. Selective medium for detecting *Pseudomonas glumae* Kurita et Tabei, the causal bacterium of grain rot of rice. Japanese Journal of Phytopathology, 52(2): 253-259. https://doi.org/10.3186/jjphytopath.52.253

Ura H, Furuya N, Iiyama K, Hidaka M, Tsuchiya K and Matsuyama N. 2006. *Burkholderia gladioli* associated with symptoms of bacterial grain rot and leaf-sheath browning of rice plants. Journal of General Plant Pathology, 72: 98-103. https://doi.org/10.1007/s10327-005-0256-6

Zhou XG. 2019. Sustainable strategies for managing bacterial panicle blight in rice. In Protecting rice grains in the post-genomic era. London, UK: IntechOpen. Pp. 67-84. https://doi.org/10.5772/intechopen.84882

Zhou-qi C, Bo Z, Guan-lin X, Bin L and Shi-wen H. 2016. Research status and prospect of *Burkholderia glumae*, the pathogen causing bacterial panicle blight. Rice Science, 23(3): 111-118. https://doi.org/10.1016/j.rsci.2016.01.007