



Review

Detection and Control Bacteria Cause Grain Rot *Burkholderia glumae* on Rice

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ABSTRACT

Burkholderia glumae, before mid-2018, is categorized as plant quarantine pest A2 Group 1 that its existence has been detected in Indonesia. *B. glumae* has been known to spread in the central production of rice in Java, Sumatra, Borneo dan Sulawesi. This review aimed to explain the strategies for *B. glumae* detection through its characteristics and to prevent the divergence of this bacterium in Indonesia. The previous studies reported that the bacteria could reduce yield up to 75% and caused the decrease of weight-grain or the increase of empty grain. The disease intensity is affected by environmental and physiological factors such as warm temperature at nighttime and high rainfall intensity. The optimum temperature for the development of the disease is 30–35°C. Moreover, the pathogen could survive at a temperature of 41°C. The tropical area of 32–36°C are suitable for *B. glumae*. Recently, the effective control of the disease in the field has not been found yet. Meanwhile, early detection of the disease is not yet determined, even though it is necessary to prevent its spread in rice cultivation in Indonesia. Detection of the disease by Agricultural Quarantine Agency as a frontline is needed to check the entry of the disease carried by the import activities of the seed. Detection in the suspected field by protection institutes through frequent surveillance in central production areas of rice should be considered as an important task. The effective techniques to prevent *B. glumae* are the use of resistant varieties, the practice of seed treatments (using antibacterial, bactericide, heat treatment or plant extract), and the application of oxolinic acid to the crops.

Keywords: *Burkholderia glumae*, control method, detection strategies, grain rot, quarantine

INTRODUCTION

The grain rot of rice, previously classified as a plant quarantine pest, is one of the serious threats to the national rice production. Handiyanti *et al.* (2018) stated that grain rot caused by *Burkholderia glumae* was detected in rice varieties from several locations in Java, i.e. IR64 from Purworejo, Ciherang from Cirebon, and Inpari Sidenuk from Banyuwangi. *B. glumae* is a plant pathogenic bacterium cause blight symptoms in rice panicles, seed rot, and wither on plants (Nghiep *et al.*, 2001; Saylor *et al.*, 2006; Kim *et al.*, 2010). Saylor *et al.* (2006) also reported that *B. glumae* is transmitted through infected seeds. *B. glumae* was first discovered in Japan as a cause of grain rot and blight on seedlings and thereby being an important disease of rice in Japan (Rush, 2007).

According to Ministry of Agriculture Regulation No. 51/Permentan/KR.010/9/2015 concerning the types of plant quarantine pests, *B. glumae* classified

into plant quarantine pest A2 Group I which means that it is already a presence in Indonesia, yet cannot be controlled by any treatment. However, after the issuance of Permentan No. 31/Permentan/KR.010/7/2018 concerning types of plant quarantine pests, *B. glumae* has been removed from the list of plant quarantine pests. This indicates that *B. glumae* has spread to central rice production in Indonesia. In addition, *B. glumae* has become an issue in various countries, i.e. Korea, Japan, the USA, and other Asian countries (Kim *et al.*, 2012). *B. glumae* distribution areas include Indonesia (Java, Sumatra, Kalimantan), Asia (China, Taiwan, Japan, Korea, Philippines, Sri Lanka, Vietnam), America (Colombia, Panama, USA), and Africa (Burkina Faso) (Peraturan Menteri Pertanian No. 51 Tahun 2015). In the southern United States, this disease caused a loss yield by 40% during 1995 and 1998, and even in some places up to 80% (Fang *et al.*, 2009; Ham *et al.*, 2011; Nandakumar *et al.*, 2009; Shahjahan *et al.*, 2000a; Zhou *et al.*, 2011).

Trung *et al.* (1993) reported that *B. glumae* can reduce rice yield by up to 75% for reducing grain weight, empty grains, and inhibition of seed germination.

Government efforts to prevent the entry of bacteria cause grain rot continues to be done through the role of the Plant Quarantine Agency. BBKP Surabaya has prevented the entry of imported Chinese seeds infected by *B. glumae* in 2014. In the following year, BBKP Soekarno-Hatta also succeeded in preventing the entry of rice seeds from China and the Philippines which were infected by *B. glumae* (Barantan, 2015). Therefore, this paper was aimed to inform about the detection technique of *B. glumae* through its characteristics, efforts to prevent the spread, and appropriate control in rice cultivation in Indonesia.

BIOECOLOGY

The scientific classification of *B. glumae* is (Ballard *et al.*, 1970; CABI, 2017):

Phylum	: Proteobacteria
Class	: Betaproteobacteria
Order	: Burkholderiales
Family	: Burkholderiaceae
Genus	: Burkholderia
Species	: <i>Burkholderia glumae</i> (Kurita & Tabei, 1967)

B. glumae is a gram-negative bacterium, which was previously known as *Pseudomonas glumae* Kurita and Tabei (Yuan, 2004; Nandakumar *et al.*, 2009; Pinson *et al.*, 2010). *B. glumae* is a non-fluorescent bacterium that produces a green-yellow color, the water-soluble pigment in various media, and rod-shaped bacteria with 1–3 flagella. In the media, bacterial colonies produce grayish-white or yellow pigments (Yuan, 2004) (Figure 1).

Grain rot caused by *B. glumae* is transmitted through seeds (Goto & Ohata, 1956; Uematsu *et al.*, 1976) and epiphytes develop in plants, especially in the maturity of rice stadia. Bacteria will develop quickly on the surfaces of newly emerged panicles and infect the flowers. The initial symptoms are small rot of 1–5 mm with brown edges on the leaves and panicles, hence caused the empty grains (Sayler *et al.*, 2006; Pinson *et al.*, 2010; Ham *et al.*, 2011). *B. glumae* produces toxoflavin toxin at a temperature of 30–38°C, which inhibit the growth of leaves and roots of rice, and chlorosis in panicles (Iiyama *et al.*, 1995; Jeong *et al.*, 2003; Suzuki *et al.*, 2004). *B. glumae* can be found in air, soil, and water. The spread of *B.*

glumae in the soil is strongly influenced by soil type, pH, cultivated plants, and weather. *B. glumae* can survive on plants during the growing season, on rice seeds stored at room temperature or even on weeds and parts of rice plants left in the field after harvesting (Yuan, 2004). Then, diseases can develop after rice plants are planted in the field. *B. glumae* are non-obligate hence they can live on the plants left in the field and they become the source of infection in the next growing season.

FACTORS AFFECTING DEVELOPMENT OF DISEASES

The severity of grain rot highly depends on weather conditions at the flowering stadia (Tsushima *et al.*, 1996). The infection will increase in high humidity and high temperatures. Several factors can affect plant susceptibility, such as the flowering duration of the variety. According to Tsushima (2011), rice plants are very susceptible to infection within 1–3 days after flowering. However, plants survive in 2 days before flowering and 4 days after flowering. In addition, plants are also susceptible to 4–5 days after the heading time to 11 days afterward. Infection rapid spread if humidity is high (> 95%), otherwise symptoms will not appear if humidity is < 70% (Tsushima *et al.*, 1995; Tshushima, 1996).

Disease development is strongly influenced by environmental conditions and plant physiology (Tshusima, 1996; Pinson *et al.*, 2010). Low temperatures inhibit disease progression and increase the development of the wax layer on the cuticle of the host plant thus it may have a role to increase plant resistance. The disease can develop rapidly in unusual environmental conditions, such as high temperatures, especially at night with heavy rain (Zeigler & Alvarez, 1990; Mew, 1992). The optimal temperature for disease development is 38–40°C (Nandakumar *et al.*, 2009) or 30–35°C (Kurita *et al.*, 1964; Tsushima *et al.*, 1986) even at 41°C (Saddler, 1994 *cit* Zhou-qi *et al.*, 2016). Thereby, the disease incidence is very high in tropical areas including in Indonesia which has an average maximum temperature of 32–36°C.

The high humidity in the flowering stadia also increases disease infection and the attack of *B. glumae* could be worse during global warming (Tsushima *et al.*, 1995; Ham *et al.*, 2011). The severity of the disease is also influenced by internal and external factors, i.e. plant susceptibility and inoculum

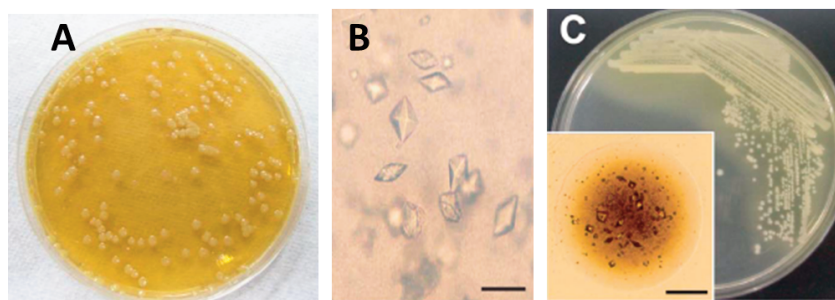


Figure 1. A and C are bacterial colonies on the media (Kim *et al.*, 2010; Niño, 2014) and B is the toxoflavin crystal produced (Kim *et al.*, 2010)

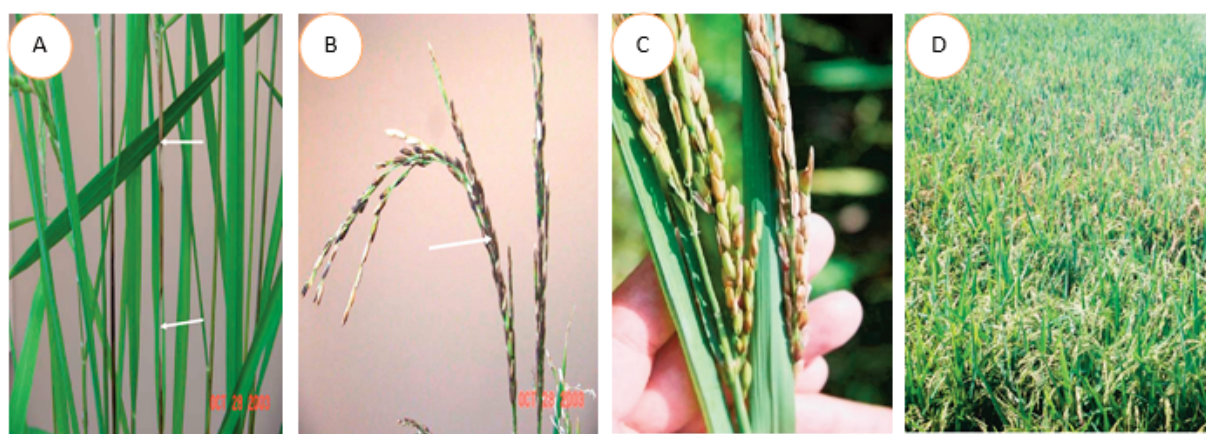


Figure 2. The symptoms of grain rot caused by *Burkholderia glumae* on the midrib of the leaves (A), rice panicle (B), panicle discoloration (C), and the disease symptoms in the field (D) (Yuan, 2004)

density of the pathogen (Tsushima, 1996). The severity of the disease will greatly increase if the rice is susceptible and very high inoculum density. According to the rainfall and temperature pattern in Indonesia, *B. glumae* will potentially spread easily in Indonesia. Therefore, early warning of the grain rot is necessitated, mainly through the use of existing climate data. The use of an integrated cropping calendar system created by the Indonesian Agency for Agricultural Research and Development can be used to determine the right cropping season, cultural practices that inhibit disease development, and the development of early disease detection in the field.

SYMPTOMS OF GRAIN ROT DISEASE

B. glumae attacks various parts of rice plants, such as leaves and grains, causes wilting or rot of the leaves (Figure 2A), and cause discoloration, empty grains, dark spots, and rot on the grains (CABI, 2017). Yuan (2004) stated that *B. glumae* can cause rot on grain, seedling, and midrib of the

rice. The specific symptom of *B. glumae* is a blight on panicles accompanied by the color-changing (usually brown-grey), the empty grain, upright panicles with straw-colored, and eventually the midrib turn gray lesion with a reddish-brown margin (Nandakumar *et al.*, 2009) (Figure 2B). These symptoms in the field are similar to the symptoms caused by stem borer and neck blast disease.

The disease can occur through primary or secondary infections. Primary infection occurs when the infected seeds are planted in the field. Meanwhile, secondary infection occurs when surrounding plants near the source of infection are infected by the disease. After infection, the panicles color will change from green to reddish-brown (Mizobuchi *et al.*, 2013). Tsushima (2011) stated that pathogens initially colonize the lower surface of the lodicule and the inner surface of the lemma. When attacking in the flowering stadia, the bacteria will cause empty grains.

DETECTION METHOD

Detection of plant diseases is necessary to determine the cause of disease hence the most effective control technique could be conducted. The accuracy of disease detection will determine the effectiveness of the used control technique. The misdetection causes control to be ineffective. The first effort to detect disease is by recognizing the symptoms of the disease. The specific symptoms can be used as an indicator to determine the pathogen. However, the symptoms appear in the field are usually similar hence difficult to distinguish. Especially if the observations are late to be done thereby the symptoms appear are caused by secondary causes. Therefore, the right time of observation is the key to knowing the symptoms caused by *B. glumae*.

For the accuracy of *B. glumae* detection, Barantan (2015) has a recommendation for testing *B. glumae* through the use of the culture method as a confirmation test method. Whereas Tsushima *et al.* (1986) have developed semi-selective media known as SP-G media. Furthermore, Kawaradani *et al.* (2000) found a selective medium for accessing *B. glumae*, this medium known as CCNT, in 1,000 ml of sterile water contains of 2 g of yeast extract, 1 g of polypepton, 4 g of inositol, 10 mg of cetrimide, 10 mg of chloramphenicol, 1 mg of novobiocin, 100 mg of chlorothalonil, and 18 g of agar. After being incubated for 2–4 days at 41°C, *B. glumae* will produce a yellowish colony on the media which is different from other bacteria species. This result is considered to be useful to ensure the cause of the disease accurately if the symptoms are difficult to distinguish.

The development of selective media is often constrained by a large number of microorganisms in nature (Andrews & Harris, 2000; Torsvik & Øvreås, 2002), thus often causing the growth of other organisms on these selective media (Alvarez, 2004). Therefore, Kawanishi *et al.* (2011) developed a selective media known as SMART (Selective Medium–design Algorithm Restricted by Two constraints). SMART media were developed not from natural ingredients that act as selectors and contain two ingredients (carbon and antimicrobials). Besides being able to detect the presence of *B. glumae*, SMART is also able to recognize the ecology and epidemiology of the target bacteria.

Kim *et al.* (2012) stated that the detection procedure for *B. glumae* bacteria could be done through the bio-PCR method. Previously, Sayler *et al.* (2006) have developed an effective PCR method for detecting *B. glumae* in seeds and in plants. PCR and real time-PCR have been used as the main method to identify several *Burkholderia* species originating from the soil, water, infected plants, and mammalian cells (Hikichi *et al.*, 2001; Furuya *et al.*, 2002; Sayler *et al.*, 2006). The PCR primer based on 16S-23S rDNA ITS is able to detect and analyze the presence of *B. glumae* without DNA extraction or agar gel electrophoresis. PCR is considered able to convert and count small amounts of bacteria–infected seeds, even though asymptomatic (Nandakumar *et al.*, 2009). The ITS method was carried out by Handiyanti *et al.* (2018) to detect the rice varieties infected by *B. glumae* in several locations in Java, the ITS primer can be used for early detection of *B. glumae* in rice seed. Detection of seeds infected by pathogens is also needed through strengthening the role of the agricultural quarantine officer at the entrance and exit of the seeds as well as the existing seed supervision and certification officer. In the field, detection and monitoring can be carried out by the Plant Pest Monitoring Officer (POPT) in each area. Detection in the field needs to be done on time to prevent the diseases spread to other plants. Therefore, the selection of the kit in detecting the disease is necessary.

CONTROL STRATEGIES AND ANTICIPATIVE STEPS

Quarantine is the first step to be implemented to prevent the spread of grain rot caused by *B. glumae*. An indication of the entry of *B. glumae* from several exporting countries certainly gives an idea to be able to strengthen the front gate of the Technical Implementation Unit of the Agricultural Quarantine Agency. Rapid detection methods need to be applied hence the type of pest infected the rice seed can be detected. In addition, the role of quarantine in preventing *B. glumae* between regions in Indonesia must be optimized. The status of *B. glumae* is become important disease of rice (OFTP), indicates that this disease has existed in several regions in Indonesia and its spread must be prevented. Quick steps are needed to determine the right control strategy in the

field thus farmers can conduct the control technique immediately if rice plants are infected with grain rot.

Research on *B. glumae* in Indonesia is still limited and only becomes the object of monitoring by the Agricultural Quarantine Agency. Therefore, when an attack occurs in the field, effective control procedures can not yet be carried out. Kim *et al.* (2012) reported that controlling *B. glumae* is very difficult to do so that only practical methods can be recommended, i.e. through the use of pathogen-free seeds, cultural practices, and the use of resistant varieties. Early detection methods need to be established by seed health test and certification agencies in each region. Therefore, in addition by having the knowledge of disease monitoring and detection in the field, the proper disease detectors are needed to be used by the field officers to detect the cause of the disease.

Control techniques to prevent the spread of disease can be done by using resistant varieties. Krausz (2005) reported that the use of resistant varieties may become a promising technique to control *B. glumae*. The results of his study showed that each cultivar has a different level of resistance against *B. glumae* infection. These results were similar to Saylor *et al.* (2006) which stated that Drew varieties and LM-1 lines are relatively resistant to *B. glumae* (Figure 3A), whereas Krausz (2005) showed that Saber and Jefferson cultivars are more resistant to *B. glumae* and BF4-274 line also has resistant characteristics (Figure 3B). The results showed that 2 weeks after inoculation, each variety had different resistance. Based on these results, the use of resistant varieties may become an alternative control technique to suppress disease attacks. However, some rice varieties produced by Balitbangtan do not

yet have specific resistant characteristics to *B. glumae*. Thereby, in the future, the breeding of resistant rice varieties in Indonesia to several plant quarantine pests, not only to control important pests, are necessitated to be done.

Several studies have shown that seed treatment is the most effective effort to prevent the attack of *B. glumae*. This was confirmed by Belmar *et al.* (2014) reported that an effective control strategy to suppress *B. glumae* is through seed treatment without reducing seed germination. According to him, several methods of seed treatment can be used, i.e. through the use of chemicals, heating technology, and seed washing (Table 1). Previously, Iwai *et al.* (2002) proved that the transfer of thionin-expressing genes from oats to rice plants was effective in suppressing *B. glumae*. Table 1 showed that several technologies can be used to control *B. glumae*. The use of garlic extract for seed treatment can be performed to suppress *B. glumae* infection. However, the reduction in the germination to be 70% is still being reconsidered. The results of the applied test conducted by Barantan (2015) also proved that the combination of hot water and bactericidal treatments can be recommended as quarantine treatment on rice seeds to eliminate *B. glumae*. The treatment of hot water at 56°C for 30 minutes followed by soaking into a copper hydroxide solution at a concentration of 2000 ppm for 60 minutes and drying at 40°C for 24 hours effectively eliminating *B. glumae* without reducing seed viability. As for applications in the field, further research is still needed to determine the pesticide efficacy to control *B. glumae*. According to Zhou-qi *et al.* (2016), spraying bactericide and treating seeds with oxolinic acid proved to be effective in

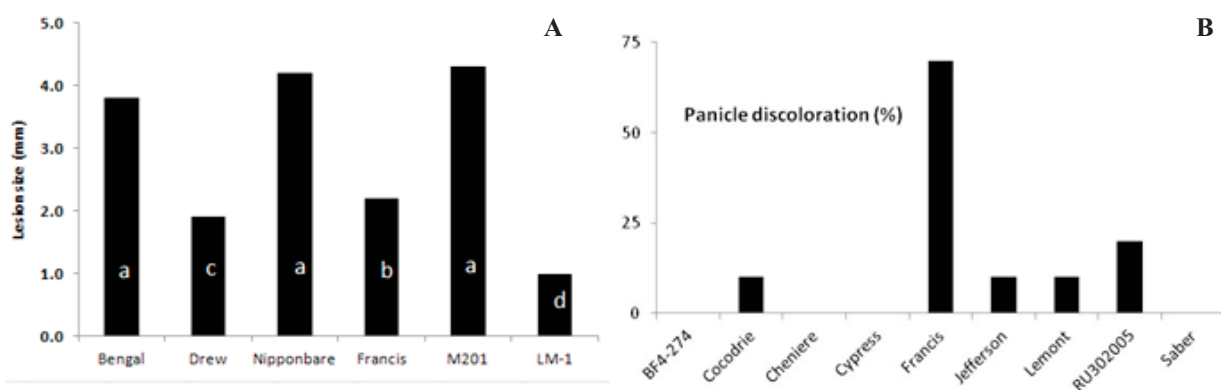


Figure 3. The lesion size on the rice (A) and panicle discoloration (B) in varying varieties and lines of rice (Krausz, 2005; Saylor *et al.*, 2006)

Table 1. Control techniques to suppress *Burkholderia glumae* in seed treatment

Control technique	Concentration/ Temperature	Time (hour)	Percentage of infected seeds	Percentage of germination
Control (<i>B. glumae</i>)	-	-	100	90
Heating	55°C	72	0	90
Hot water treatment	55°C	0.025	15	50
Cold temperature	-20°C	48	100	0
Vinegar	2%	72	45	86
Klorox + heating at 52°C	-	0.17	18	90
Garlic extract	1.5 ml/3 g seed	1	30	70

Source: Belmar *et al.* (2014)

suppressing *B. glumae*. But later according to him, this chemical leads the resistant strains of *B. glumae*.

CONCLUSION

Early detection of *B. glumae* is needed as an effort to prevent the spread of disease in rice plantations in Indonesia. Detection of *B. glumae* can be done through observing symptoms and identifying pathogenic characteristics through the use of selective media and PCR technology. Detection on the source of the disease can be conducted by the Agricultural Quarantine Agency as the front guard to detect the process of entry of rice seeds infected by *B. glumae*, originating from abroad or from regions in Indonesia. Detection in the field can be done through routine monitoring in several centers of rice cultivation areas that have indicated the presence of pathogens. Strengthening the ability of the field to be able to overcome the problem of disease attack in the field is also necessary to prevent disease development. The most effective control procedures for suppressing grain rot has not yet been found. Some efforts to suppress the disease can be done through the use of resistant varieties, seed treatment, heating technology, and the use of plant extracts that are known to be able to suppress *B. glumae* infections. As for applications in the field, it can be done by spraying bactericide or treating seeds with oxolinic acid. However, further studies are still needed to be able to apply by the farmers.

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