Rapid detection of *Burkholderia glumae* causal agent of grain rot disease in rice seed from Gowa Regency, South Sulawesi using ELISA

A A Darmawan, T Kuswinanti and A Asman

Plant pest and Disease Department, Agriculture Faculty, Hasanuddin University, Makassar 90245, South Sulawesi, Indonesia

E-mail: koeswinanti@yahoo.com

Abstract. Bacterial grain rot in rice plants caused by *Burkholderia glumae* becomes a serious threat because it is a seed borne pathogen that causes severe damage to rice plantations in several regency at South Sulawesi. This study aimed to rapidly detect of *B. glumae* causal agent of grain rot disease in rice seed from Gowa Regency, South Sulawesi. Samples were obtained in 10 districts in the main rice producing areas of Gowa regency, South Sulawesi. Sampling using Non Probability Sampling method. 10 Samples that showing grain rot symptoms were detected using Indirect ELISA test with monoclonal antibody. The detection results shown that 9 out of 10 districts are confirmed positive for *B. glumae* bacteria.

1. Introduction

Rice (*Oryza sativa* L) becomes the main agricultural commodity in Indonesia. Bacterial grain rot caused by *Burkholderia glumae* is one of important disease because it can reduce rice yields [1]. This seed borne pathogenic bacteria [2, 3] attacks rice plantations causing 40% losses [3] and severe infection reported up to 80% [4]. *B. glumae* was first reported in Japan in the 1950s [5, 6] and it spread to the other countries of Asia, Africa, and America [7-10]. Importation of seeds, global climate change, and cultivation practices are thought related to outbreak this disease [11].

In Indonesia, bacterial grain rot disease was first reported in 1987 but the damage was still insignificant, but after that the damage due to this disease has not been reported again [11] until 2015, the presence of this disease began to be reported again in several regency of South Sulawesi [12]. The bacteria has been detected in South Sulawesi at several regency that is Maros, Barru, Sidrap, and Luwu [13]. There has no reported about this bacteria in Gowa regency. However, Gowa regency has suitable for the development of this disease because prolonged high night temperatures in Indonesia has suitable to environment for outbreaks of grain rot disease [11, 14]. Therefore, it is necessary to detect the existence of *B. glumae* causal agent of grain rot in rice seed from Gowa regency, South Sulawesi.
2. Methods

2.1. Sampling
Samples obtained in 10 districts in the main rice producing areas of Gowa regency, South Sulawesi using Non Probability Purposive Sampling method. Samples that obtained showing grain rot symptoms.

2.2. Prepare of the test
Detection of bacteria B. glumae using Indirect-ELISA test with monoclonal antibody for B. glumae from Agdia Inc. 1X buffers were prepared and it will be used for sample. Samples were crushed using a mortar and pestle to extract the bacteria.

2.3. Test procedure
Total of 0.5 grams each one of the sample were crushed then mixed with 100 μl of 1X coating buffer with ratio of 1:10 (weight/volume). Positive and negative controls of B. glumae were prepared and then all of the samples were added into the microplate and incubated in a humid box lined with wetted tissue at 4°C for 1 night.

Blocking solution of 5% nonfat dry milk in PBS buffer were prepared and then 200 μl of blocking solution were added to each well. After that plate incubated in a humid box for 30 minutes at 37°C. Antibody preparation carried out within 10 minutes before use by diluting concentrated PBST buffer with nonfat dry milk with ratio according to antisera kit instructions. After blocking incubation is complete, plate washed using a quick flipping motion to dump the wells into a waste container without mixing the contents. All the wells filled completely with 1X PBST, and then wells quickly emptied again and repeated twice. After washing, frame were holded by upside down and tapped firmly on a folded paper towel to remove all droplets of wash buffer.

Detection antibody were added by dispense 100 μl of prepared detection antibody per well and then plate incubated in a humid box for 1 hour at room temperature. Then, enzyme conjugate preparation carried out within 10 minutes before use. Preparing the enzyme conjugate diluent by dilute concentrated PBST buffer with nonfat dry milk with ratio according to the manual reagent used. All the wells filled completely with 1X PBST, and then wells quickly emptied again and repeated 7 times. After washing, frame were holded by upside down and tapped firmly on a folded paper towel to remove excess liquid from the frame.

Enzyme conjugate were added by dispense 100 μl of prepared enzyme conjugate per well and then plate incubated in a humid box for 1 hour at room temperature. About 15 minutes before the end of the above incubation step, 5 ml of room temperature 1X PNP buffer measured for each tablet that will be used. Then without touching the tablets, PNP tablets added to the buffer. The PNP tablets and the PNP solution should not be touched or exposed to strong light. Light or contamination could causing background color in negative wells. Wells emptied into a waste container. All the wells filled completely with 1X PBST, then wells quickly emptied again and repeated 7 times. After washing frame were holded by upside down and tapped firmly on a folded paper towel to remove excess liquid from the frame.

PNP substrate were added by dispense 100 μl of PNP substrate into each testwell. Plate incubated in dark place for 60 minutes. Examining the wells by measuring on a plate reader at 405 nm. Sample indicated as negative if average absorbance value less than 2x average absorbance value of negative control. Sample indicated as positive if average absorbance value more than 2x average absorbance value of negative control.

3. Results and discussion
Total of 10 samples were obtained from 10 main rice producing areas of Gowa regency, South Sulawesi. The varieties that found in the field including Inpari 32, Ciperang, Ciliwung, and Ciguelis. All of the samples shown brown spot or necrotic lesions symptoms which appear from base of the grains and in severe infections, panicle erect and loss of grain weight (figure 1) due empty grains with a dark basal rot (figure 2).
Detection of *B. glumae* bacteria was using Indirect-ELISA test with monoclonal antibody for *B. glumae*. Afterward, read the absorbance value which was using ELISA reader at 405 nm wavelength. Based on result, it was shown that the sample from South Bontonompo district has an average absorbance value was 0.426 and 2x of average absorbance value of negative control was 0.550, which means that sample has less than 2x average absorbance value of negative control (table 1) so sample indicated as negative. The other 9 samples were Pattallassang, Parangloe, Pallangga, Bontomarannu, Somba Opu, Barombong, West Bajeng, Bajeng, and Bontonompo district has an average absorbance value of more than 2x average absorbance value of negative control so the sample indicated as positive. The highest average absorbance value of the samples was Bontomarannu district and the lowest average absorbance value of the samples was South Bontonompo.

Table 1. ELISA absorbance values at 405 nm wavelength.

| No. | Samples code | Varieties | Place of origin | Absorbance values | Average | Result |
|-----|--------------|-----------|-----------------|-------------------|---------|--------|
| 1.  | Control (+)  | -         | -               | R<sub>1</sub> = 0.479  R<sub>2</sub> = 0.520 | 0.500   | +      |
| 2.  | Control (-)  | -         | -               | R<sub>1</sub> = 0.276  R<sub>2</sub> = 0.273 | 0.275   | -      |
| 3.  | PLIn         | Inpari 32 | Pattallassang   | R<sub>1</sub> = 2.927  R<sub>2</sub> = 3.025 | 2.976   | +      |
| 4.  | PLCh         | Ciherang  | Parangloe       | R<sub>1</sub> = 3.018  R<sub>2</sub> = 3.030 | 3.024   | +      |
| 5.  | PgCh         | Ciherang  | Pallangga       | R<sub>1</sub> = 2.335  R<sub>2</sub> = 2.284 | 2.310   | +      |
| 6.  | BmCh         | Ciherang  | Bontomarannu    | R<sub>1</sub> = 3.080  R<sub>2</sub> = 3.076 | 3.078   | +      |
| 7.  | SOCh         | Ciherang  | Somba Opu       | R<sub>1</sub> = 1.685  R<sub>2</sub> = 1.930 | 1.808   | +      |
| 8.  | BrCh         | Ciherang  | Barombong       | R<sub>1</sub> = 2.491  R<sub>2</sub> = 2.435 | 2.463   | +      |
| 9.  | BBCh         | Ciherang  | West Bajeng     | R<sub>1</sub> = 1.253  R<sub>2</sub> = 1.328 | 1.291   | +      |
| 10. | BSCw         | Ciliwung  | South Bontonompo| R<sub>1</sub> = 0.428  R<sub>2</sub> = 0.423 | 0.426   | -      |
| 11. | BjCw         | Ciliwung  | Bajeng          | R<sub>1</sub> = 3.024  R<sub>2</sub> = 3.115 | 3.070   | +      |
| 12. | BnCg         | Cigeulis  | Bontonompo      | R<sub>1</sub> = 1.203  R<sub>2</sub> = 1.144 | 1.174   | +      |

The symptoms that found in the field were symptoms of bacterial grain rot disease. Symptoms of bacterial grain rot disease were reddish-brown necrotic lesions starting from the base of the grains [10,
and heavily infected panicles remained upright due to blank and also show aborted seeds with a dark basal rot [16].

Based on the ELISA result, the sample from South Bontonompo district indicated as negative and samples from Pattallassang, Parangloe, Pallangga, Bontomarannu, Somba Opu, Barombong, West Bajeng, Bajeng, and Bontonompo indicated as positive. The sample that has negative results was suspected that the symptoms of rice grain rot can also be caused by B. gladioli or B. plantarii, which symptoms were similar and arduous to the difference between species based on the symptoms found in the field. The presence of B. gladioli in Japan saw grain rot of rice symptoms [17]. B. glumae and B. gladioli were the pathogens causing rice grain rot, which was showing similar symptoms and it was not possible to distinguish between species in the field [10]. B. glumae, B. plantarii, and B. gladioli are also known to infect rice plants causing similar symptoms [18].

4. Conclusion

B. glumae the bacterial causal agent of grain rot disease in rice seed has been detected in 9 out of 10 districts in Gowa Regency, South Sulawesi including Pattallassang, Parangloe, Pallangga, Bontomarannu, Somba Opu, Barombong, West Bajeng, Bajeng, and Bontonompo. Infected rice varieties including Inpari 32, Ciberang, Ciliwung, and Cigeulis.

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