Morphological characterization of several strains of the rice-pathogenic bacterium *Burkholderia glumae* in North Sumatra

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**Abstract.** *Burkholderia glumae* is a quarantine seed-borne bacterial pathogen causing panicle blight disease on rice. This pathogen has been detected in some locations in Java, and recently, farmers in North Sumatra have reported rice yield loss with symptoms similar to those on rice infested by the rice-pathogenic bacterium *B. glumae*. This research was aimed to isolate several bacterial strains from several rice varieties in various locations in North Sumatra and characterize the morphology of the strains to detect and identify the unknown bacterial strains presumably *B. glumae*. Several rice seed varieties were collected from Medan and Deli Serdang Districts. The seed samples were extracted, isolated and purified, then grown in semi-selective media PPGA. The morphological characteristics of the bacterial strains were determined including Gram staining, bacterial colony’s and bacterial cell’s morphology. The results showed that of eleven strains isolated, two strains were Gram negative and nine strains were Gram positive. On the basis of colony morphology, all strains had circular form, flat elevation and cream colour while the colony margin varied, i.e. entire and undulate. Most strains had bacillus/rod shape (8 strains) and only 3 strains were coccus.

1. Introduction

Rice (*Oryza sativa* L) is the main staple food for more than two million people worldwide, mostly live in Asia including in Indonesia. Rice contains high carbohydrate for human’s source of energy. Of the total rice production in the world, i.e. 490.6 million tons [1], more than 90% is produced and consumed in Asia. Rice consumption of Indonesian people has ranked as the first position in the world which resulted in high rice demand [2].

Rice production in Indonesia is forecast to decline in 2016 [1]. In North Sumatra, rice production has been fluctuated with 4.06 million tons in 2015, which is slightly increase 11.40% compared to 2014 [3].

A number of factors affect the decrease of rice production in Indonesia including un-remunerative prices, unseasonable weather, lack of good quality of seed and pest and diseases [1]. Bacterial panicle blight (BPB) on rice was first reported in Japan in 1950s and the disease has been one of serious plant diseases in the world [4]. BPB has been reported in rice producing countries including Southern and Middle America (Dominique Republic, Venezuela, Equador, Brazil, Panama, Columbia, Nicaragua and Costarica), Southern Africa and Tanzania, Asia (Japan, Korea, Vietnam, Phillipines, India, Indonesia, Malaysia, Thailand and China) [5]. Rice panicle blight can cause yield losses up to 75% in
several states in the USA and more than 40% crop losses in Panama and several tropical and sub-tropical countries have considered BPB as potential risk disease on rice [6,7].

Recent report from Dr. Suryo Wiyono, a lecturer at Bogor Agricultural Institute, confirmed the occurrence pathogen of B. glumae in hybrid rice seeds in Tegal (Middle Java) and Blitar (East Java), although Ministry of Agriculture of Indonesia denied his statement [8]. Similarly, BPB has been detected in Southern Sulawesi [9]. In Indonesia, panicle blight was a minor rice disease since 1987, however no report causing significant crop losses after that [10]. Indonesian government has listed B. glumae as a quarantine pathogen (Group I, Category of A2), which cannot be removed from the carrier through quarantine treatments [11]. This disease has become an emergence disease that affects rice production in several locations in Indonesia since 2015 [12]. The infected rice plants produce empty panicle which causes crop failure. Recently, similar symptoms have been found in several rice fields in North Sumatera and have caused serious concerns to the farmers. As there is no information and research about this disease in North Sumatera, therefore research on detection and identification of this disease is urgently required. This research was aimed to characterize the morphology of several bacterial strains isolated from several symptom and symptomless rice seeds in North Sumatera. Bacterial morphology is one aspect of bacterial identification.

2. Materials and methods
2.1 Rice seed samples
Rice seed samples were collected from several places in North Sumatra. 90 g of the seeds were placed in a polyethylene plastic bag and labelled, then brought to the Laboratory of Plant Disease, Faculty of Agriculture, University of Sumatera Utara for further analysis.

2.2. Seed extraction, isolation and purification
The seeds sample were extracted, isolated and purificated to obtain pure cultures on semi-selective media PPGA and King’s B media according to Safni et al. [13] and IRRI [14] with modification. The seeds were surface-sterilization with NaClO 0.5% for three minutes to sterilize the pathogens on the seed surface.

2.3. Morphology test
2.3.1. Cellular morphology
Cellular morphology of the bacteria included the cell shape which was observed under the microscope and Gram-staining. Gram-staining test was performed according to Schaad et al. [15]. Bacterial cell shape and color was observed using microscope with 1,000 times magnification. Gram-negative bacteria is red or pink color while Gram-positive bacteria expose blue or purple color.

2.3.2. Colony morphology
The colony morphology was determined after 48 hours of incubation on the basis of colony’s color, shape, elevation and margin according to Bergey’s Manual of Systematic Bacteriology [16].

3. Results and discussion
3.1. Rice seed samples
From four different locations, eleven bacterial strains were collected (Table 1). Eight strains were isolated from Deli Serdang and three strains were isolated from Medan.
Table 1. Bacterial strains from several rice varieties isolated from two locations

| No | Bacterial strains | Location   |
|----|-------------------|------------|
| 1  | IR64 M            | Medan      |
| 2  | CH MD             | Medan      |
| 3  | CH M              | Medan      |
| 4  | IP30              | Deli Serdang |
| 5  | IP32              | Deli Serdang |
| 6  | ME                | Deli Serdang |
| 7  | SIB               | Deli Serdang |
| 8  | CIB               | Deli Serdang |
| 9  | CID               | Deli Serdang |
| 10 | IR64 SR           | Deli Serdang |
| 11 | CH SR             | Deli Serdang |

3.2. Morphology test

Morphology results of cell shape, Gram staining and colony’s morphology tests are displayed in Table 2. It showed that only 5 strains were Gram negative, IR64 M isolated from Medan and ME, SIB, IR64 SR and CH SR isolated from Deli Serdang. The rest strains (6) were confirmed as Gram positive bacteria. The cell shape majority was rods/bacillus (8 strains) and only 3 strains were coccus. The colony’s color, shape and elevation were similar for all strains, i.e. cream, circular and flat respectively. For colony’s margin varied with entire margin for 5 strains and undulate margin for 6 strains.

According to Brenner et al. [16] in Bergey’s Manual of Discriminative Bacteriology, B. glumae is characterized as rods/bacillus, 0.5-0.7 x 1.5-2.5 μm, Gram negative. On the basis of these characteristics, the possible bacterial strains of B. glumae are strains of IR64 M isolated from Medan, and SIB isolated from Deli Serdang.

For further work, biochemical characteristics as well as molecular essay are required for bacterial identification.

Table 2. Morphology of bacterial strains isolated from rice seeds

| No | Bacterial strains | Cell shape/Arrangement | Size             | Gram-staining | Colony’s morphology | Margin |
|----|-------------------|------------------------|------------------|---------------|---------------------|--------|
|    |                   |                        |                  |               | Color              | Shape  | Elevation |             |        |
| 1  | IR64 M            | Bacillus               | 0.69 μm x 0.59 μm | -             | Cream              | Circular| Flat      | Undulate   |        |
| 2  | CH MD             | Bacillus               | 3.24 μm x 0.74 μm | +             | Cream              | Circular| Flat      | Undulate   |        |
| 3  | CH M              | Bacillus               | 2.29 μm x 0.67 μm | +             | Cream              | Circular| Flat      | Undulate   |        |
| 4  | IP30              | Bacillus               | 2.53 μm x 0.61 μm | +             | Cream              | Circular| Flat      | Entire     |        |
| 5  | IP32              | Bacillus               | 3.13 μm x 0.78 μm | +             | Cream              | Circular| Flat      | Entire     |        |
| 6  | ME                | Coccus                 | 0.85 μm x 0.45 μm | -             | Cream              | Circular| Flat      | Entire     |        |
| 7  | SIB               | Bacillus               | 1.076 μm x 0.48 μm | -             | Cream              | Circular| Flat      | Entire     |        |
| 8  | CIB               | Bacillus               | 3.24 μm x 0.66 μm | +             | Cream              | Circular| Flat      | Undulate   |        |
| 9  | CID               | Bacillus               | 2.43 μm x 0.86 μm | +             | Cream              | Circular| Flat      | Undulate   |        |
| 10 | IR64 SR           | Coccus                 | 1.03 μm x 0.39 μm | -             | Cream              | Circular| Flat      | Entire     |        |
| 11 | CH SR             | Coccus                 | 0.93 μm x 0.42 μm | -             | Cream              | Circular| Flat      | Undulate   |        |

4. Conclusions

It is concluded that on the basis of morphological characteristics of eleven bacterial strains isolated from several rice varieties and two districts in North Sumatra, one strain isolated from Medan (IR64 M) and one strain isolated from Deli Serdang (SIB) are presumably confirmed as B. glumae, the causal bacterial pathogen of rice panicle blight.
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Acknowledgement
The authors gratefully acknowledge that this research was supported by Grant of TALENTRA 2017, Universitas Sumatera Utara, Indonesia (Contract number 285/UN5.2.3.1/PPM/KA-CREASEUS/2017).