Control of bacterial leaf blight and rice blast with antagonist microbial in the agroecosystem of irrigated rice

A B Pustika*, K Yolanda and Sudarmaji
Assessment Institute of Agricultural Technology of Yogyakarta, Jl. Stadion Maguwoharjo No. 22 Ngemplak Sleman DI. Yogyakarta, Indonesia.

Corresponding author: arlynabudi@gmail.com

Abstract. Bacterial leaf blight (Xanthomonas oryzae) and rice blast (Pyricularia oryzae) cause yield losses in rice production. Antagonist microbial application through seed treatment and foliar spray during rice growing stage is suggested to prevent the disease which is more reliable to reduce the disease incidence than curing it. This research aimed to determine the severity of bacterial leaf blight and rice blast among varieties that treated with antagonist microbial complex in the agro ecosystem of irrigated rice. Research was conducted in Kalibawang sub district, Kulon Progo, Yogyakarta, October 2018 to January 2019. The experimental design was randomized block design with 7 treatments and 4 replications. The treatments were the application of antagonist microbial complex formula A (Azotobacter vinelandii, Azospirillum sp., Bacillus cereus, Bradyrhizobium sp., and Methylobacterium sp. >10^7 CFU/g) and formula B (Azotobacter chroococum, Azotobacter vinelandii, Azospirillum sp., Pseudomonas cepacia, Penicillium sp., and Acinetobacter sp. >10^7 CFU/g) to several rice varieties (Inpari 9, Inpari 33, and Ciherang). Results shows that formula B had the lowest severity of bacterial leaf blight (14.44%) and leaf blast (6.94%) at Ciherang while formula A had the lowest panicle blast (6.55%). The highest yield (10.62 t/ha) was obtained from Ciherang treated with formula B.

1. Introduction
Rice (Oryza sativa L.) is a staple food consumed by about 90% of Indonesia's population. Indonesia rice production in 2019 was 54 million ton and targeted to increase 7% per year. The requirement for rice in Indonesia remains to increase as the population growth. Rice field area was 7.463 million hectares and harvested as 10.677 million ton. With rice productivity 5.114 t/ha, target of husked rice production in 2020 is 39.2 million ton to feed 268 million people with addition of 1.5–2 million ton rice surplus. Yogyakarta has 111 thousand hectares of harvested rice area, produced 533 thousand ton of rice and 301 thousand ton of husked rice in 2019. Yogyakarta rice productivity was 4.786 t/ha [1]. Rice productivity can still be improved in various ways with the technical improvement in cultivation.

Rice is susceptible to several diseases caused by bacteria and fungi. Bacterial leaf blight (BLB) caused by Xanthomonas oryzae pv. oryzae (Xoo) and blast caused by Pyricularia oryzae are the most serious diseases that resulted poor crop growth and yield loss [2]. Bacterial leaf blight is a vascular disease resulting a systemic infection of susceptible rice plant. The disease manifests tannish-grey to white lesions along the veins and most often observed at the tillering stage called kreas. Then the leaves of the entire plant turn pale yellow and wilt. Research shows that young plants (3 weeks old) are more susceptible. Rice blast severely affects the quantity and quality of rice in most of current rice growing areas [3,4]. Pyricularia oryzae causes infection in panicle, neck and leaf. Leaf blast is the most damaging
stage for yield losses in the rice field [5-7]. Blast disease spreads mostly through airborne conidia through the year. In early infection stage, the interfering hyphae produce in host cells leading to the progress of primary symptoms i.e., eye shaped spots [8-12]. Main infection is seed borne while secondary infection occurs due to spread of spores by numerous means.

Bacterial leaf blight caused yield reduction of 20–50% at the tiller stage in severe cases, while rice blast is responsible for large yield losses [13-15]. This disease is an important problem in several countries, including Indonesia. The yield losses caused by BLB reached 70%–80% in Indonesia, 74%–81% in India, and 20%–50% in Japan [16]. BLB occurrences cause crop loss, reaching 21%–36% during rainy season and 18%–28% in the dry season [17]. Rice blast is one of the most damaging rice diseases of its widespread and destructive nature, making yield losses up to 60-65% in vulnerable rice varieties [18]. The fungus may infect any above portion of rice plants, including roots and seeds. It also revealed that there is a systemic movement of the pathogen from seed to seedlings [19]. The disproportionate use and misapplication of the synthetic pesticides in disease control have raised serious questions regarding their long-term effects on human health, soil quality, and the environment [20]. Biological control is supposed as an environmentally approachable and cost effective substitute to chemical control. There are studies on application of bacterial antagonist to Xanthomonas oryzae [21]. Many of these bacteria are native to the rice rhizosphere. The most common samples are rhizobacterial fluorescent pseudomonads, which are to be antagonistic to many bacterial and fungal diseases [22,23]. There are also reports about Bacillus which have been effectively inhibit bacterial leaf blight, Paenibacillus polymixa and Streptomyces that can control bacterial leaf blight and rice blast [24,25].

Antagonist microbial can be engaged as seed treatment before sowing, root dips prior to transplanting and foliar sprays. Control of diseases through bio control agents have become a need of time and being investigated in the recent era [26]. In several studies, application of antagonist microbial resulted lower disease incidence as well as higher grain yields. Currently there are many microbial formulation products which is contain of antagonist microbial complex enriched with root nodules bacteria (Bradyrhizobium japonicum) and formulated in liquid form. The formulation is produced through biotechnology processes to support the needs of environmental friendly farming to control disease as well as increase crop production [27]. This study was aimed to determine disease severity of bacterial leaf blight and rice blast among varieties applied with antagonist microbial complex in the agro ecosystem of irrigated rice.

2. Methods
Research was conducted in Kalibawang sub district (7°43'37", 110°12'51"), Kulon Progo, Yogyakarta, from October 2018 to January 2019. Rice varieties planted in this research were Inpari 9, Inpari 33, and Ciherang. Antagonist microbial complex applied to the rice varieties were Formula A and Formula B. Formula A was antagonist microbial complex A, contained of Azotobacter vinelandii, Azospirillum sp., Bacillus cereus, Bradyrhizobium sp., and Methylobacterium sp. >10^7 CFU/g. Formula B was antagonist microbial complex B, contained of Azotobacter chroococcum, Azotobacter vinelandii, Azospirillum sp., Pseudomonas cepacia, Penicillium sp., and Acinetobacter sp. >10^7 CFU/g. Formula A (500 g) was applied to 25 kg rice seed as seed dressing before sowing. Formula B (10 ml) was suspended into 1 litre of water, then applied to rice seed as seed dressing before sowing. Four weeks after transplanting, Formula A was sprayed with dosage 300 g/ha, while Formula B was sprayed with concentration of 5 ml/l and dosage 1.5 l/ha with spray volume 300 l/ha. The experimental design was randomized block design with 7 treatments and 4 replications. The treatments were as follow:

1) Inpari 9 applied with Formula A (antagonist microbial complex A)
2) Inpari 33 applied with Formula A (antagonist microbial complex A)
3) Ciherang applied with Formula A (antagonist microbial complex A)
4) Inpari 9 applied with Formula B (antagonist microbial complex B)
5) Inpari 33 applied with Formula B (antagonist microbial complex B)
6) Ciherang applied with Formula B (antagonist microbial complex B)
7) Ciherang without antagonist microbial
The development of bacterial leaf blight, leaf blast and panicle blast were observed at two weeks after transplanting and continuously recorded with interval 14 days. Disease severity of bacterial leaf blight, leaf blast and panicle blast were determined using Standard Evaluation System (SES) for rice [28]. In addition to disease severity, yield data was recorded by measuring grain weight based on Teknik Ubinan Jajaer Legowo Super [29].

Diseases severities were calculated using the formula:

\[
DS = \frac{\sum (n \times v)}{N \times V} \times 100\%
\]

Where:
- \( DS \) = Disease severity
- \( n \) = Number of leaves infected
- \( v \) = Value score of each category attack
- \( N \) = Number of leaves observed
- \( V \) = Value of the highest score

All data were analysed for treatment effect differences by Analysis of Variance (ANOVA). Comparison of means was performed using Duncan Multiple Ranged Test (DMRT) (p=0.05) by the SAS statistical package.

3. Results and discussion

3.1. Disease severity of bacterial leaf blight

The effect of antagonist microbial complex against bacterial leaf blight was presented in Figure 1. Disease severity of bacterial leaf blight began to increase at 6 weeks age after transplanting until 10 weeks. Although formula B (the antagonist microbial complex B) controlled bacterial leaf blight tend to be better than formula A, the effect to the disease severity is not significantly different. Formula B lead to lower disease severity at Inpari 9, Inpari 33 and Ciherang in 10 weeks age as 15.00%, 14.44% and 14.44% respectively which is not significantly different from application of formula A at Inpari 9, Inpari 33 and Ciherang of 15.83%, 16.94% and 16.39% respectively. However, disease severity at plants applied with antagonist microbial complex was significantly lower than disease severity at plants without application of antagonist microbial complex with result of 17.50% in Ciherang variety. From the result, seed treatment and foliar application of antagonist microbial complex in Formula A which contain Azotobacter vinelandii, Azospirillum sp., Bacillus cereus, Bradyzhizobium sp., and Methyllobacterium sp. >10^7 CFU/g and formula B which contain Azotobacter chroococum, Azotobacter vinelandii, Azospirillum sp., Pseudomonas cepacia, Penicillium sp., and Acinetobacter sp. >10^7 CFU/g are resulting reduction of disease severity against bacterial leaf blight.

Other research showed seed treatment with Pseudomonas fluroscens suspension resulted in reductions of rice bacterial leaf blight. In addition, seed treatment also increased seed germination and seedling vigour of rice varieties [30]. The antagonistic fluorescent pseudomonas produces one or more metabolites, such as phenazine-1-carboxylic acid, DAPG, pyoluteorin, pyrrolnitrin (PRN), and oomycin. DAPG is a polyketide antibiotic with a broad spectrum of antimicrobial properties. The inhibition of Pseudomonas is through the production of some siderophores like pseudobactin, pyochelin, pyoveradine, ferribactin, ferrichrome, ferrooxamine, phytosiderophorous etc., antibiotics like phenazines, pyoluteorin, tropolone, pyocyanine,2,4-diacetyl phologlucinol (DAPG), pyrrolnitrin. It also produces secondary metabolites like Hydrogencyanide, phenazine-1-carboxylic acid (PCA), oomycin A, indole-3-acetic acid, chitinase, β-1,3 glucanase, laminariase. With the production of many antibiotics, siderophores and other toxins Pseudomonas fluroscens acts as an effective and broad spectrum antagonistic spectrum [31]. Other research found that Bacillus sp. is a gram-positive bacterium which produces antimicrobial or surfactant compounds and enzymes as lipase against pathogenic bacteri and fungi [32]. Bacillus pumillus from a rice field was characterized as an antagonistic against Rhizoctonia solani, which is also known to produce antifungal metabolites to inhibit mycelial growth as well as spore germination of a number of pathogenic fungi as Aspergillus and Penicillium species [33-
All these rhizosphere bacteria potentially act as both biological control agent and biofertilizer [36,37]. Seed treatment of rice seed before sowing using endophytic bacteria consortium contain of Bacillus sp., Burkholderia sp., Citrobacter sp., Klebsiella sp., and Pseudomonas aeruginosa to control BLB was also reported [38]. Successful biocontrol against rice pathogen growing demand for sound, biologically based pest management practices and suggesting that the market potential of biocontrol products will increase in coming years [39].

### Figure 1. Disease severity of bacterial leaf blight in several rice varieties treated with antagonist microbial complex

#### 3.2 Disease severity of leaf blast.

The effect of antagonist microbial complex in the development of leaf blast symptom was shown in Figure 2. The lowest disease severity of leaf blast (6.67%) was occurred at Ciherang applied with formula B. However, low severity of this disease was also presented by Inpari 33 and Ciherang applied with formula A with result 7.50% and 6.94% respectively, as well as Inpari 33 applied with formula B (6.94%). The result suggested both formula A and B are very useful to control leaf blast disease in Inpari 33 and Ciherang. At the other side, the disease severity in Inpari 9 could not be reduced by the application of formula A and B, it resulted high disease severity (25.00% and 21.67% respectively). It was surprisingly that Inpari 9 seemed not responsive to the seed dressing and foliar application of antagonist microbial complex. The susceptible genetic response of Inpari 9 against Pyricularia oryzae become a possible reason for this finding. The resistance gene for leaf blast can be race and non-race specific, meaning it is often overcome by the pathogen, which in turn directed to high levels of infections variability in cultivation areas [40,41]. Moreover, more virulent populations of the pathogen develop and resistant cultivars lose their effective resistance within a certain time period [42]. The incorporation of resistant genes was a continuous process to counter progressing pathogens allowing to the gene-for-gene hypothesis [43]. It was considered pathogenic variation among strains results an essential role in
rice blast dynamic and thus in the achievement of integrated disease control, particularly for breeding of resistant rice varieties. Therefore, genetic variability among *Pyricularia oryzae* (*M. grisea*) pathotypes should be engaged into concern when using it for rice genotypes screening of blast resistance [44].

![Figure 2: Disease severity of leaf blast in several rice varieties treated with antagonist microbial complex](image)

**Figure 2.** Disease severity of leaf blast in several rice varieties treated with antagonist microbial complex

### 3.3. Disease severity of panicle blast

Infected panicles appear as white and are unfulfilled partially or totally, causing seed failure to fill, or causing the whole panicle to fall over as it rots. The lesions are often grayish brown discoloration of panicle branches which may break at the lesions over time [45]. The panicles become white when young necks are infected and later infection caused incomplete grain filling and poor grain quality [46]. The influence of antagonist microbial complex against panicle blast disease severity was revealed in Figure 3. Development of disease was increased from week 8th to week 12th after transplanting. Although had been seed dressed and sprayed with antagonist microbial complex, Inpari 9 presented highest disease severity (16.39% – 17.50%) than Inpari 33 (6.39% - 9.44%) and Ciherang (5.56% - 7.78%). This result is in line with the high severity of leaf blast in Inpari 9 compares to Inpari 33 and Ciherang. It is suggested that susceptible genetic response of Inpari 9 against *Pyricularia oryzae* was the main factor. Formula A seemed most effective to reduce panicle blast in Ciherang (5.56%) while formula B lead to lower panicle blast severity in Inpari 33 (6.39%). However, the effect between formula A and B was not different in reducing panicle blast both in Inpari 33 and Ciherang.
Figure 3. Disease severity of panicle blast in several rice varieties treated with antagonist microbial complex

3.4. Yield of rice
The highest yield (10.62 t/ha) was obtained from Ciherang variety applied with formula B, followed by Ciherang applied with formula A (10.40 t/ha). Inpari 33 applied with formula A produced 8.18 t/ha, while thus applied with formula B gained 9.88 t/ha. The lowest yield was presented by Inpari 9 applied with formula A (7.09%), followed by thus applied with formula B (7.95%). The result on yield indicated that antagonist microbial complex (formula A and B) not only reduced the disease severity in Inpari 33 and Ciherang, but also increased the yield. It is well recognized that antagonist microbial complex promote plant growth and decrease the disease severity caused by fungal pathogens [47]. Formula A and B contain microbial complex such as *Bacillus* spp., *Pseudomonas* spp., and *Actinomycetes* spp. which have been evaluated for their capability to biocontrol rice blast in the field [48-51], prevent the growth of *Pyricularia oryzae* mycelia and suppress diseases in vitro [52,53]. *Bacillus* spp. are categorized by an ability to biocontrol many diseases and pests, improve the soil nutrient condition, stimulate plant growth, yield and stress resistance. It also performing as microbial fertilizers, repairing contaminated soil, and degrading cellulose, and are applied extensively for sustainable agricultural development. *Bacillus* spp. colonize rice plants and compete with plant pathogens for colonization sites and nutrients. This bacteria also produce antimicrobial metabolites that inhibit the growth hyphae and conidia of pathogen. Moreover, *Bacillus* spp. soften the cell wall of pathogen and produce enzymes to decrease disease severity [54-58].

4. Conclusion
Results showed that the application of antagonist microbial complex was apparent to reduce bacterial leaf blight and blast. Formulation B, the antagonist microbial complex B lead to the lowest severity of bacterial leaf blight (14.44%) and leaf blast (6.67%) at Ciherang. Formulation A lead to the lowest
panicle blast (5.56%) at Ciherang. Although antagonist microbial complex A and B were effective against bacterial leaf blight and panicle blast, they revealed lower effect on leaf blast reduction in Inpari 9, hence their effect on leaf blast should be reassessed under similar field condition in endemic areas. Reduction of disease severity resulted higher yield in Ciherang (10.62 t/ha).

References
[1] BPS 2020 Statistical Year Book of Indonesia (Jakarta: Badan Pusat Statistik)
[2] Suryadi Y, Susilowati D N, Kadir T S, Zaffan Z R, Hikmawati N and Mubarak N R 2013 Asian Journal of Plant Pathology 7 92–108
[3] Fisher M C, Henk D A, Briggs C J, Brownstein J S, Madoff L C, McCraw S L and Gurr S J 2012 Nature 484 186–94
[4] Kihoro J, Bosco N J, Murage H, Ateka E and Makihara D 2013 SpringerPlus 2 308
[5] Mousanajad S, Alizadeh A and Safaie N 2010 Journal of Agricultural Science and Technology 12 357–64
[6] Pasha A, Babaeian-Jelodar N, Bagheri N, Nematzadeh G and Khosravi V 2013 International Journal of Agriculture and Crop Sciences 5 390–4
[7] Liu Q, Yang J, Zhang S, Zhao J, Feng A, Yang A T, Wang X, Mao X, Dong J, Zhu X, Leung H, Leach J E and Liu B 2016 Mol. Plant Microbe Interact. 29 46–56
[8] Wilson RA and Talbot N J 2009 Reviews Microbiology 7 185–95
[9] Valent B and Khang C H 2010 Curr. Opin. Plant Biol. 13 434–41
[10] Giraldo M C and Valent B 2013 Nature Reviews Microbiology 11 800–14
[11] Liu W, Liu J, Ning Y, Ding B, Wang X, Wang Z and Wang G L 2013 Molecular Plant 6 605–20
[12] Zhang H, Wu Z, Wang C, Li Y and Xu J R 2014 Nat. Commun. 5 4518
[13] Kim K H, Cho J, Lee Y H and Lee W S 2015 Afric. For. Meteorol. 203 191–207
[14] Ni D, Song F, Ni J, Zhang A, Wang C, Zhao K and Li L 2015 Field Crops Res. 184 1–8
[15] Ji Z, Yang S-D, Zeng Y-X, Liang Y, Yang C-D and Qian Q 2016 J. Integrative Agric. 15 1432–40
[16] Djamikho HA, Prakoso B and Prihartiningsih N 2011 Jurnal Hama dan Penyakit Tumbuhan Tropika 1 35–46
[17] Suparyono and Sudir 1992 Media Panel. Sukamandi 6 6–12
[18] Chandrasekhara M V, Gururaj S, Naik M K and Nagaraju P 2008 Karnatak J. Agril. Sci. 21 305
[19] Manandhar H K, Jorgensen H J L, Jorgensen S B and Petersen V S 1998 Plant Disease 82 1093–9
[20] Ramlı@Yusof N H, Yusup S, Kueh B W B, Kamarulzaman P S D, Osman N, Rahim M A, Aziz R, Mohktar S and Ahmad A B 2018 Sustainable Chemistry and Pharmacy 7 1–8
[21] Chae J C, Hung N B, Yu S M, Lee H K and Lee Y H 2014 J Microbiol Biotechnol. 24 740–7
[22] Velusamy P, Immanuel J E, Gnanamanickam S S and Thomashow L 2006 Can. J. Microbiol. 52 56–65
[23] Lingaiah S and Umesha S 2013 Canadian Journal of Plant Protection 1 147–53
[24] Chen X, Wang G, Xu M, Jin J and Liu X 2010 African J. of Microbiology Research 4 2692
[25] Chung E J, Hossain M T, Khan A, Kim K H, Jeon C O and Chung Y R 2015 Plant Pathol J. 31 152
[26] Naqvi SAH 2019 Pakistan Journal of Agricultural Research 32 359
[27] Baharuddin R, Jahuddin A, Yani and Tuwo M 2019 IOP Conf. Series: Earth and Environmental Science 355 012080
[28] IRRI 2013 Standard Evaluation System for Rice 5th edition (Manila: IRRI)
[29] Balai Besar Penelitian Padi 2010 Teknik Ubinan Pendugaan Produktivitas Padi Menurut Sistem Tanam (Subang: Balai besar Penelitian Tanaman Padi)
[30] Ramanamma and Santoshkumari M 2017 International Journal of Current Microbiology and Applied Sciences 5 124
[31] Karthikeyan A, Parthasarathy R and Manikandan A 2013 *J. Microbiol. Biotech. Res.* **3** 77
[32] Fritze D 2004 *Phytopathology* **94** 1245
[33] Munimbazi C and Bullerman L B 1998 *J. Appl. Microbiol* **84** 959
[34] Padaria J C, Srivastav S and Singh A 2008 *J. Eco-Friendly Agriculture* **3** 56
[35] Padaria C and Singh A 2009 *Journal of Environmental Science and Health* **44** 397
[36] Nontji M and Amran FD 2019 *Makara Journal of Science* **23** 87
[37] Nontji M and Amran FD 2019 *Makara Journal of Science* **23** 87
[38] Fritze D 2004 *Phytopathology* **94** 1245
[39] Munimbazi C and Bullerman L B 1998 *J. Appl. Microbiol* **84** 959
[40] Suryadi Y, Susilowati DN, Kadir TS and Ruskandar A 2012 *Jurnal Agrotropika* **17** 7
[41] Sreevongchai T, Toojinda T, Thanintorn N, Kosawang C, Vanavichit A, Tharreau D and Sirithunya P 2009 *Plant Breeding* **129** 176
[42] Shahriar S A, All Imtiaz A, Hossain M B, Husna A and Eaty M KN 2020 *Annual Research & Review in Biology* **35** 50
[43] Thaku RP, Shetty KG and King SB 1992 *Plant Pathology* **41** 626
[44] Flor HH 1971 *Annual Review of Phytopathology* **9** 275
[45] Bonman J M 1992 *Euphytica* **63** 115
[46] Shafaullah K, Khan M A, Khan N A and Ysir MNK 2020 *Annual Research & Review in Biology* **35** 50
[47] Sreevongchai T, Toojinda T, Thanintorn N, Kosawang C, Vanavichit A, Tharreau D and Sirithunya P 2009 *Plant Breeding* **129** 176