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Endophytic bacteria: an emerging tool for biological control bacterial leaf blight of paddy

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Abstract. Endophytic bacteria were isolated from the root of paddy, not negative influence even could serve as plant growth promoter and pathogen biocontrol likely *Xanthomonas oryzae* pv. *oryzae* caused of bacterial leaf blight. The research was aimed to know the effectivity of controlling the endophytic bacteria against bacterial leaf blight of paddy. Methods of this research were suppressing effect to control bacterial leaf blight with seed dipping and soaking the endophytic bacterial suspension on surrounding plant in the polybag. Nine bacterial and one control were treatment in this assay and three replicates, arranged with randomize completely block design. Endophytic bacteria isolated from roots of paddy were evaluated for their capacity to suppress bacterial leaf blight intensity, infection rate and the effectivity control. The result showed that the endophytic bacteria could suppress the disease, and the isolate of SB1 (from Sumbang 1) is the best for antagonistic effect by 64.16% and 49.14% effectivity at screen house and paddy fields respectively. Infection rate of this disease was slow by 0.024 unit.day⁻¹ and different with the control was 0.088 unit.day⁻¹.

1. Introduction

Sustainable management system of agriculture are key tools to maintain yield over the years, in such systems plants might be protected from diseases or other adversities with environmentally friendly tools that have low impact on the production and also on the environment. As a consequence, the interest for biological control of plant pathogens is increasing and strategies of biological control have been proposed and developed [1,2] as well as biopesticide formulations containing endophytic bacteria [3]. In addition, varieties with resistance against diseases [4] and efficient agronomic management were also adopted to control pathogens. In this scenario, biocontrol emerges not only as a reliable alternative to chemical pesticides but it also may provide control of diseases that cannot be managed by other strategies, such as in the case of phytopathogenic bacteria [5] and endophytic bacteria, providing opportunities for a rationale and safe crop management.

Protection of plants from pathogens can be achieved either through an antagonistic interaction or by activating mechanisms such as the induced systemic resistance [6,7]. Among the microorganisms that can protect plants against pathogens are the endophytes [8]. These microorganisms inhabit plant intercellularly and are therefore less exposed to environmental stresses than the rhizobacteria, so endophyte is potential as biocontrol agents [9]. Recently it has also been shown that they enhance



plant growth and health [10,11], although they could be potential biocontrol agents of diseases by antagonizing bacterial and fungal plant pathogens [8].

Xanthomonas oryzae pv. *oryzae* is the pathogen of bacterial leaf blight of paddy that a main disease constrains production of staple crop in many countries of the world. Identification of *X. oryzae* pv. *oryzae* (Xoo) was conducted based on the disease symptom, pathogenicity, morphological, physiological, and genetic characteristics of bacterial cultures isolated from the infected plants. The characteristics of Xoo is aerobic, rod shaped, and Gram negative. The isolates were evaluated for their hypersensitivity in tobacco and pathogenicity in rice plants. Their isolates induced hypersensitive reaction in tobacco and showed pathogenicity symptom in paddy in different length. The symptom of bacterial leaf blight showed generally infects leaves, but distribution depends highly on its environmental conditions (12). Infection usually starts from tip of the leaf and spreads all the way to the base. Symptoms that occur in paddy during its vegetative phase are called *kresek*, and in its generative phase they're called blight (13,14). If pathogen attacks the plant during the seed phase, its leaves will wither, curl, and become grayish-green in color. If attack occur on mature plants, the leaves will turn pale yellow. Xoo has the potential to reduce yield up to 50% or more depending on the variety, stage of the crop and climatic conditions.

Endophytic bacteria are bacteria that living at internal tissues of plant (root, stem, leaf, flower), and doesn't cause symptoms in plants, and is even beneficial effect on their host because of it can increase plant growth by being able to produce compounds that are secondary metabolites such as antibiotics, enzymes, and others [15]. Endophytic bacteria activity is a tool that directly or indirectly affects plant growth and health. The direct activity of endophytic bacteria as pathogenic biocontrol is evidenced by the mechanism of antibiosis by producing siderophore, HCN, enzymes, and other compounds that are antibacterial or antifungal. The capability to colonize internal tissue make endophytic bacteria can against pathogens by mechanism of competitive site and nutrient or produce antagonistic substance [16].

The penetrate of endophytic bacteria to internal tissues of the plant is connected by their capability to produce cellulase [17]. Controlling of bacterial leaf blight with endophytic bacteria from paddy healthy root had prospects for tools in plant disease management. The indirect mechanism of the endophytic bacterial was to promote plant growth and induce systemic resistance of plants against some pathogens. The objective of this research was to know the effectivity of controlling the endophytic bacteria against bacterial leaf blight of paddy.

2. Methods

The research was conducted in two steps namely in screen house and paddy fields. The assay of endophytic bacteria in screen house conducted with artificial inoculation by clipping methods, and in paddy fields by natural inoculation of Xoo because the bioassay at an endemic area of bacterial leaf blight.

2.1. Bioassay for the effectiveness of endophytic bacteria to control bacterial leaf blight at the screen house

Eight endophytic bacteria from healthy paddy root and one control tested for suppressing bacterial leaf blight conducted in screen house. The experiment was arranged with Completely Randomized Design with nine treatments and three replicated. The treatments were K: control, B1: Endophytic bacteria (EB) from Karangwangkal 5 + Xoo; B2: EB Karangwangkal 7 + Xoo; B3: EB Karangwangkal 8 + Xoo; B4: EB Sumbang 1 + Xoo, B5; EB Sumbang 3 + Xoo; B6: EB Serayu 5 + Xoo; B7: EB Serayu 7 + Xoo; B8: EB Somagede 1 + Xoo. The application of endophytic bacteria by seed dressing over night, and spraying at 20 and 30 days after transplanting with population density is 10^9 cfu/mL. The inoculation Xoo by clipping method at 30 days after transplanting on polybags with population density of Xoo was 10^9 cfu/mL. Variable observed were incubation periods, disease intensity, infection rate and effectivity control. The disease intensity was observed with formula by (18, 19), $DI = \{\sum(n \times v) / ZxN\} \times 100\%$, DI: disease intensity, n: number of plant in each symptom category, v: category score

number, Z : the highest category, N: number of observed plant. The categories of symptoms are: 0: no symptom, 1: 1-5 square, 2: 6-10 square, 3: 11-15 square, 4: 16-20 square, 5: 21-25 square. One square means 4 mm². The Incubation period observed if the first symptom appears. Infection rate calculate by (20) $X_t = X_o.e^{rt}$, and r was infection rate that calculate by *simple interest disease* value was $r = 2,3/t \{ \log 1/(1-X_t) - \log 1/(1-X_o) \}$, r: infection rate, X_t : proportion of symptom at t time and X_o : proportion of symptom at previously appear. The effectivity control of endophytic bacteria against bacterial leaf blight was found by compare with disease intensity of treatment and the control. Data was analyzed by anova and if significant effect than continued by DMRT 5%.

2.2. Bioassay for the effectiveness of endophytic bacteria for control bacterial leaf blight at the paddy fields

The result of bioassay at the screen house, and the selection endophytic bacteria from laboratory assay, the selective isolates were three endophytic bacteria namely SM1 isolates from Somagede 1, SB1 from Sumbang 3 and SB3 from Sumbang 3, that their isolates used to paddy fields bioassay to suppressing bacterial leaf blight disease. The experiment arranged with Randomized Completely Block Design four treatments and six replicated. The treatments were A: control, B: Endophytic bacteria (EB) from Somagede 1 (SM1); C: EB Sumbang 1 (SB1); D: EB Sumbang 3 (SB3). The application of endophytic bacteria by seed dressing over night, and spraying at 20, 30, and 40 days after transplanting with population density is 10⁹ cfu/mL. The inoculation Xoo by natural inoculation (in endemic location of bacterial leaf blight). Variable observed were incubation periods, disease intensity, infection rate, and effectivity control, with the same formula previously at the screen house assay. Data was analyzed by anova and if significant effect than continued by BNT 5%.

3. Results and discussion

3.1 Bioassay for the effectiveness of endophytic bacteria to control bacterial leaf blight at the screen house

Table 1. Pathosystem component of BLB after endophytic bacterial application in screen house

Treatment	Incubation Period (dai)	Disease Intensity (%)	Effectiveness (%)
Control	3	24.22 a	0
B1	3	12.66 b	47.73
B2	4	14.08 b	41.87
B3	5	13.86 b	42.77
B4	4	8.68 c	64.16
B5	5	13.26 b	45.25
B6	4	15.18 b	37.32
B7	3	15.02 b	37.98
B8	6	12.28 b	49.29

Notes: The same letter after the value in same colom showed not significant different by DMRT 5%. K: control, **B1**: Endophytic bacteria (EB) from Karangwangkal 5 + Xoo; **B2**: EB Karangwangkal 7 + Xoo; **B3**: EB Karangwangkal 8 + Xoo; **B4**: EB Sumbang 1 + Xoo, **B5**: EB Sumbang 3 + Xoo; **B6**: EB Serayu 5 + Xoo; **B7**: EB Serayu 7 + Xoo; **B8**: EB Somagede 1 + Xoo.

The disease intensity of bacterial leaf blight at the screen house showed a decrease in the application of endophytic bacteria. Table 1 showed that all of endophytic bacteria can suppress the bacterial leaf blight, their potential effect significant different with control. The incubation period in range 3-6 days after inoculation. Sumbang 1 (B4) isolate had the best effectiveness in controlling bacterial leaf blight with disease intensity and control effectiveness of 8.68% and 64.16% respectively. This effectiveness is caused by the isolates from suboptimal area where the high of Fe and Al which are endophytic bacteria isolated from healthy plants among the symptomatic plant around them so that endophytic

bacteria can produce siderophores which are used to chelate Fe into siderophore-Fe compounds which available for plant. Pathogens have less Fe, so their growth and infection process were inhibited. The occurrence Fe^{3+} deficiency required by pathogens because of Fe^{3+} is bound by siderophore [21]. Apart from that iron is an important element in the development disease, so that the binding of iron by siderophore then the pathogen is less able to infect, so inhibits disease progression [22].

3.2 Bioassay for the effectiveness of endophytic bacteria to control bacterial leaf blight at the paddy fields

The assay of endophytic bacteria at paddy fields showed in Table 2 that incubation periods 30 days after transplanting on control and a delay of symptoms appear until 42 days after transplanting on C treatment (SB1 isolate). The disease intensity at paddy fields showed a significant difference between control and three endophytic bacterial treatments. Sumbang 1 isolate is the best control to reduce the disease intensity of bacterial leaf blight. This result showed by plant growth and yield were better than other treatments. The endophytes treated paddy plots showed a significantly lower intensity of bacterial leaf blight (17,180%) compared to untreated control plots (33,778%), which also recorded a higher grain yield. Endophytic bacteria that identification by (23) as the *B. subtilis* (FZB 24) treated rice plants registered higher induction of defense related enzymes, peroxidase, polyphenol oxidase and phenylalanine ammonia lyase and resulted in higher accumulation of total phenols compared to untreated control plants. The endophytes treated rice plots registered a significantly lower intensity of bacterial leaf blight (2.80%) compared to untreated control plots (19.82%), which also recorded a higher grain and straw yield. Endophytic bacteria can reduce the intensity of bacterial leaf blight and promote plant growth by increase in IAA production, number of tillers, and leaf size (24).

Table 2. Pathosystem component of BLB after endophytic bacterial application in paddy fields

Treatment	Incubation period (dat)	Disease intensity (%)	Infection rate (unit.day ⁻¹)	Effectiveness (%)
A: Control	30	33.778 a	0.088	0
B: SM1	38	24.212 b	0.038	28.32
C: SB1	42	17.180 c	0.024	49.14
D: SB3	40	23.762 b	0.040	29.65

Notes: The same letter after the value in same column showed not significant different by BNT 5%

The progress curve of bacterial leaf blight showed in Figure 1, that control one was the highest intensity in every time observation. This evidence indicated that endophytic bacteria can support plants resistance to disease, which explains by their produce the peroxidase, phenol total, and other enzymes for biochemical defense of plants.

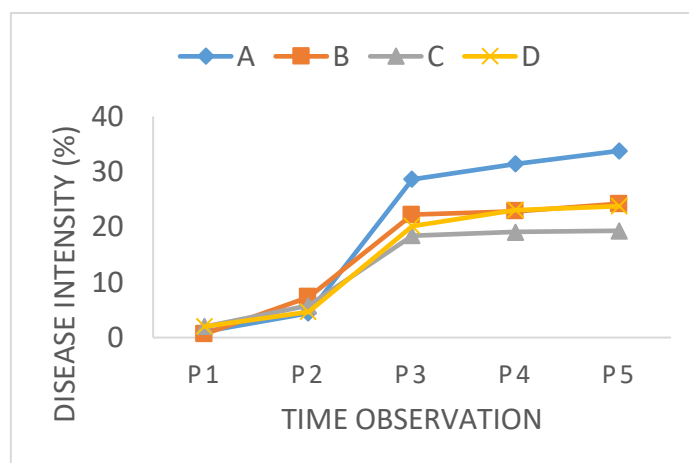


Figure 1. Disease progress curve of bacterial leaf blight on paddy fields

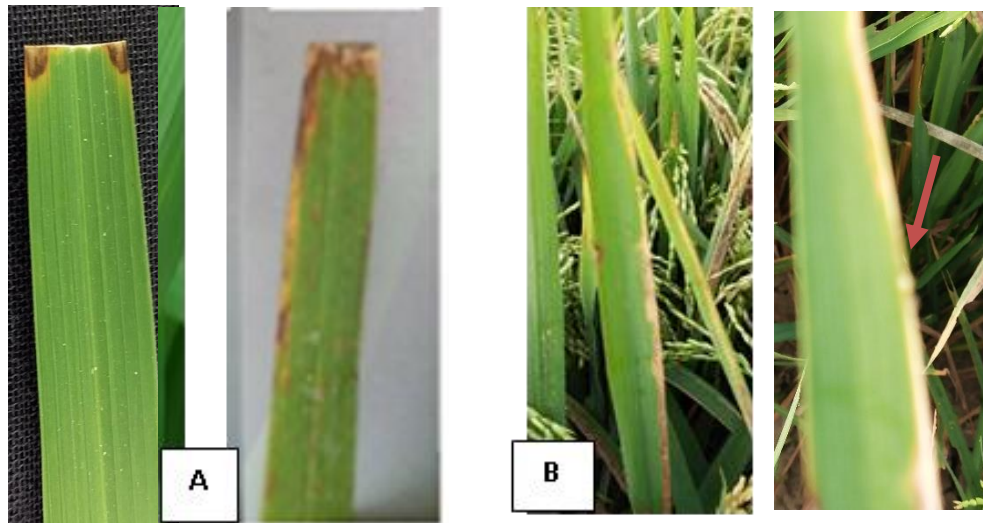


Figure 2. Symptom of bacterial leaf blight on screen house (A) and paddy fields (B)

Disease syndrome of bacterial leaf blight showed in Figure 2 (A) three days after inoculation the first symptom was appeared by yellow to brown necrosis and then the end of observation the necrosis was expanding along with the leaves. In the paddy fields, the symptom of bacterial leaf blight showed wider and yellow to greys. In the morning observation showed some ooze bacteria Xoo like drop on surface leaves (Figure 2B).

4. Conclusion

The endophytic bacteria could suppress the disease, and the isolate of SB1 (from Sumbang) is the best for antagonistic effect by 64,16% and 49,14% effectivity at screen house and paddy fields respectively. The infection rate of this disease was slow by $0.024 \text{ unit.day}^{-1}$ and different with the control was $0.088 \text{ unit.day}^{-1}$.

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