Potential of endophytic bacteria as plant growth promoter and antagonist against pineapple-fungal plant pathogen in Indonesia

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SUMMARY

This study was aimed to investigate potential of 15 endophytic bacteria (3C, AK, CH, GKSKK, GBSH, AM, B1, GKSKC, GBSK3, GKSKW, GKSKP, A31, GKSKKn, NS and AP) that were isolated from healthy leaves of pinneaple as plant growth promoter and antagonist of pineapple-fungal plant pathogens. The isolates were investigated on their hipovirulence, ability as plant growth promoter and ability to inhibit three pineapple-fungal pathogens, namely *Phytophtora* sp., *Curvularia* sp. and *Thielaviopsis* sp. The result showed that 10 out of 15 isolates were hipovirulent. Among 10 hipovirulence isolates, 7 isolates had potential as plant growth promoter (3C, AK, GKSKK, AM, B1, GKSKC and GBSK3). In the case of their antagonistic capability, the isolates which were produced the highest percentage of inhibition against *Phytoptora* sp. and *Curvularia* sp. were GKSCK at 72,48% and 66,08% of inhibition, respectively. Meanwhile, the highest percentage inhibition against *Thielaviopsis* sp. was obtained by CH at 64,82% of inhibition. In this study, we found that some of the endophytic bacteria can be plant growth promoter or antagonist or both as plant growth promoter and antagonist.

Introduction

Pineapple is one of the most important fruit comodities in Indonesia. Recently, although not very significant, pineapple production in Indonesia continues to decline. This is due to the decreased of soil fertility and infection of some plant pathogens. Three important plant pathogens that have been reported causing severe economic losses are *Phytophtora* sp., *Curvularia* sp. and *Thielaviopsis* sp.. Application extra chemicals fertilizer and fungicides to solve the problems can cause more severe harms to the environment and future cultivation and efforts to improve pineapple production. Thus, it need to find altemative methods that is safe to be used to solve the complications, and one of which are reducing the chemicals and using bio agents.

Endophytic bacteria is one of the promising bio agents that can be used to improve production of cultivated plants, including pineapple. Endophytic bacteria is bacteria that live internally inside the plant tissue, can be isolated from the plant after surface desinfection and does not cause negative effects on plant growth (Wilson, 1995; Gaiero et al., 2013). It has been reported that endophytic bacteria has capability as plant growth promoter (Gaiero et al., 2013; Santoyo et al., 2016), plant resistance inducer against plant diseases (Costa et al., 2013; Lanna-Filho et al., 2013; Yi et al, 2013; Egamberdieva et al., 2017; Leiwakabessy et al., 2018) and antagonist of many kinds of plant pathogens (Duffy and Defago, 1999; Gaiero et al., 2013).

Fifteen endophytic bacteria were successfully isolated from healthy leaves of pineapple. However, study on their potential as agricultural bio agents has not been performed. This study was conducted in order to investigate virulence, ability as plant growth promoter and antagonist of the fifteen isolates of above mentioned endophytic bacteria against three pineapple fungal pathogens, namely *Phytophtora* sp., *Curvularia* sp., and *Thielaviopsis* sp..

Material and Method

Endophytic bacteria. As much as 15 isolates of endophytic bacteria used in this study. All the strains were isolated from healthy leaves of pineapple.

Hypovirulence test. Hypovirulence test was performed using method developed by Worosuryani (2005). Sprouts of cucumber were used as indicator. Inoculation each of endophytic bacteria was repeated 3 times. Observation was performed until 14 days after inoculation. Disease severity Index (DSI) was calculated using formula : (∑N/Z); N : total score of disease severity on each individu, Z : total individu used. Score of the disease severity that was used can be explained as follows: 0 : healthy, there was no infection on hypocotyl; 1 : one or two brown spot observed with <0.25 cm of diameter; 2 : brown spot observed with < 0.5 cm of diameter with <10% of wetness area of hypocotyl; 3 : brown spot observed with > 1 cm of diameter with 10%<x<100% of wetness area of hypocotyl; 4 : black spot observed, wilt and sprouts death. The endophytic bacteria with DSI <2 was put in the group of hypovirulent bacteria.

Investigation on its capability as plant growth promoter. Cucumber plant was used as indicator plant. Investigation was conducted using methods developed by Worosuryani (2005). As much as 10 ml of bacterial suspension (~10^8 CFU/ml) was pured into planting medium of plant indicator. Inoculation each of the bacteria was repeated 3 times. Observation of plant height was performed every two days. Greenish leaves level was conducted once at 16 days after inoculation using chlorophyll content meter CCM 200 plus (opsi science) at the 3 of leaves position i.e. top, midle and bottom. Weight of wet and dry of shoot and root was conducted at 21 days after inoculation. In the case of dry weight of shoot and root, the fresh shoot and root were put into envelope and it was incubated at 80^{o}C for 3 days. After incubation, it was weight using digital balance EG 4200-2NM (Kern).

Antagonistic capability agains pineapple fungal-plant pathogens. Three pineapple fungal plant pathogens used in this study i.e. *Phytophtora* sp., *Curvularia* sp., and *Thielaviopsis* sp.. Antagonistic test was performed by scraping the bacteria using inoculating loop with a distance of 2 cm from the edge of petridish (diam 9 cm) contains Potato Sucrose Agar (PSA) medium (Potato extract 1000 ml, Sucrose 20 g, Agar 20 g) in both side. One culture of 7 old days of each of the fungal pathogens (diam 0.5 cm) was placed in the midle of petridish. As control, one culture of each of plant pathogens was put in the midle of petridish contains PSA medium without any endophytic bacteria. All the petridish were incubated at room temperature. Observation was conducted at 1, 3, 5 and 7 days after inoculation on the wide of fungal colony that was measured in milimeter. Percentage of inhibition was calculated using formula : [L1-L2/L1]x100%. L1: wide of fungal colony without endophytic bacteria, L2 : wide of fungal colony with endophytic bacteria.

Result and Discussions

In this study, 15 endophytic bacteria was investigated on their hypovirulence, capability as plant growth promoter and antagonist against 3 pineapple plant pathogens, namely *Phytophtora* sp., *Curvularia* sp. and *Thielaviopsis* sp.. The result showed that 10 out of 15 isolates showed hypovirulent (Table 1). Among those 10 hipovirulent isolates, 7 isolates showed potential as plant growth promoter. Application of the bacterial isolates resulting better growth on indicator plant compared to the untreated plants. Application of endophytic bacteria consistently improve plant height, greenish leaves, wet and dry weight of shoot and root and root length (Fig 1). Gaiero et al (2013) and Santoyo et al. (2016) stating that endophytic bacteria also could promote growth of their host plant. The bacteria release phytohormones (Bloemberg & Lugtenberg, 2001) that can improve plant growth such as 1-aminocyclopropane-1-carboxylate (ACC) deaminase (Gaiero et al., 2013; Santoyo et al., 2016), jasmonate, indole acetic acid, and abscisic acid (Patten and Glick, 2002; Forchetti et al., 2007). Beside their ability as growth promoter, endophytic bacteria was also reported as plant resistance inducer (Romeiro et al., 2005; Lanna-Filho et al., 2013). Application of endophytic bacteria has been reported can improve plant resistance against plant diseases such as bacterial leaf spot of pepper (Yi et al, 2013), bacterial leaf spot of tomato (Lanna-Filho et al., 2013), bacterial leaf blight of rice (Leiwakabessy et al., 2018), damping off on

Table 1 Disease severity index resulted by inoculation of the bacterial isolates on cucumber sprouts and its role as plant growth promoter

Isolates	Disease Severity Index	Role as plant growth promoter
AP	2.75	Not tested
GKSKKn	2.58	Not tested
NS	2.50	Not tested
A31	2.50	Not tested
GKSKP	2.42	Not tested
3C	2.00	Yes
AK	1.92	Yes
CH	1.83	No
GKSKK	1.67	Yes
GBSH	1.67	No
AM	1.33	Yes
B1	1.17	Yes
GKSKC	1.00	Yes
GBSK3	0.75	Yes
GKSKW	0.33	No
Kontrol	0.00	-

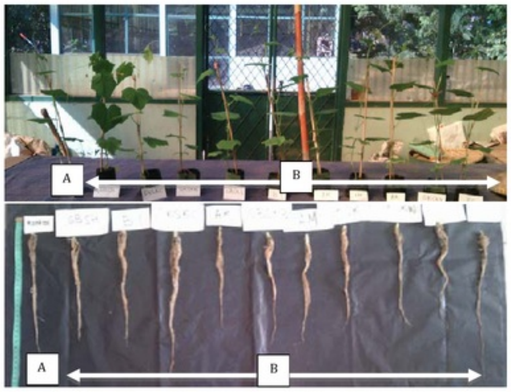

Fig.1 Indicator plant after application of bacterial isolates. A. control, B. treated plants

cucumber (Costa et al., 2013) and root rot of chickpea (Egamberdieva et al., 2017). Some of endophytic bacteria have also been reported produce anti microbial compounds, such as siderophore and antibiotics, that can inhibit growth of plant pathogens (Duffy and Defago, 1999; Gaiero et al., 2013) such as Enterobacter, *Pseudomonas* sp., *Bacillus* sp.. (Muzzamal et al., 2012), *Fusarium oxysporum* f.sp. *lycopersici* (Shahzad et al., 2017), *Phytophtora capsici*, *Alternaria panax* and *Botrytis cinerea* (Paul et al., 2013).

In this study, we found that some of endophytic bacteria used had capability to inhibit *Phytophtora* sp., *Curvularia* sp. and *Thielaviopsis* sp.. (Fig. 2). Inhibition was in the range of 5.13 to 72.48% (*Phytophtora* sp.), 2.33 to 66.08% (*Curvularia* sp.) and 1.33 to 64.82% (*Thielaviopsis* sp.). The best capability to inhibit *Phytophtora* sp., and *Curvularia* sp. was produced by GKSCK. Meanwhile, the highest inhibition of *Thielaviopsis* sp. was produced by CH (Table 2). It was shown that one endophytic bacteria can inhibit more than one pathogens.

Ability of endophytic bacteria to inhibit more than one kinds of pathogens have also been reported. Endophytic bacteria isolated from potato (Berg et al., 2005) and chilli pepper (Paul et al., 2013) have been proven to be antagonist of more than one kinds of pathogens.

Study performed by Berg et al. (2005) revealed that endophytic bacterium isolated from potato could inhibit *Verticillium dahliae* or *Rhizoctonia solani*. Paul et al. (2013) stated that edophytic bacteria isolated from chilli pepper can inhibit *Fusarium oxysporum* or *Alternaria panax* or *Colletotrichum acutatum* or *Phytophtora capsici* or *Botrytis cinerea*.

Conclusion

In conclusion, not all endophytic bacteria used in this study were plant growth promoter and antagonist. There was endophytic bacteria that play a role as plant growth promoter or antagonist or both plant growth promoter and antagonist. The best inhibition to *Phytoptora* sp. and *Curvularia* sp. were produced by GKSCK, meanwhile, the highest inhibition against *Thielaviopsis* sp. was obtained by CH.

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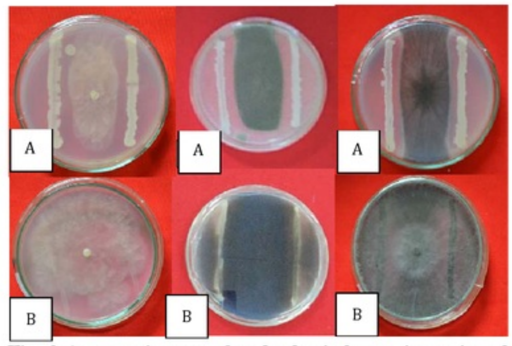

Fig. 2 Antagonist test of endophytic bacteria against 3 pineapple fungal pathogens 7 days after inoculation. From left to right : *Phytophtora* sp., *Curvularia* sp., *Thielaviopsis* sp.. A. Endophytic bacteria that had antagonistic capability, B. Endophytic bacteria that did not has antagonistic capabilitycapability.

Table 2 Percentage of inhibition of endophytic bacteria against thee pineapple fungal pathogens

Isolates	Percentage of inhibition (%)		
	Phytophtora	Curvularia	Thielaviopsis
AP*	14.76	30.18	5.58
GKSKKn*	0.00	27.77	1.53
NS*	0.00	16.73	5.44
A31*	61.85	13.35	46.15
GKSKP*	62.74	6.37	61.85
3C*	0.00	15.40	63.71
AK	64.30	29.94	4.94
CH	67.15	2.40	64.82
GKSKK	0.00	30.53	25.87
GBSH	20.00	14.48	29.68
AM	0.00	5.67	1.50
B1	0.00	9.40	8.72
GKSKC	72.48	66.08	4.88
GBSK3	5.13	2.33	0.00
GKSKW	64.11	24.88	1.33
Kontrol	0.00	0.00	0.00

* Isolates which were virulent (DSI>2) on the result of hypoverulence test

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