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The exploration of *Bacillus* spp. as antagonist agents against *Xanthomonas axonopodis* pv. *glycines* from the weed phyllosphere in soybean plantation

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ABSTRACT

Bacterial pustules caused by *Xanthomonas axonopodis* pv. *glycines* (Xag) is one of the important diseases in soybean plants. Bacillus bacteria from the soybean phyllosphere have the potential to inhibit these pathogens. Weed phyllosphere in soybean plantations is also a good habitat for Bacillus life. The purpose of this study was to obtain Bacillus from the weed phyllosphere which has the potential as an antagonistic agent against Xag. The study methods included exploration, screening, and physio-biochemical identification. The results obtained 31 isolates and 22 of them were able to inhibit Xag with various inhibitory properties. Five strains of *Bacillus* spp. had large inhibitory effects against Xag, namely strain Bp 2(2), Jg3(3), Bg d1(1), Jg 1(3) and Jg 1(4)1. The *Bacillus* strain Bp2(2) had the largest inhibition zone witht 15 mm and strain Jg1(4)1 had the fastest colony growth with 68 mm. Five *Bacillus* spp had different growth capability based on the environmental condition and carbon source. The physio-biochemical identification results indicated that *Bacillus* strain Jg 3(6), Bg d 1(1), Jg 1(3), Jg 1(4)1 had the similar characteristics to *B. licheniformis*, while strain Bp 2(2) had the similar characteristics to *B. coagulans*.

Keywords: *Bacillus*; phyllosphere; weeds; *Xanthomonas axonopodis* pv. *glycines*.

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INTRODUCTION

The soybean requirement in Indonesia annually increases as soybean is the main source of plant proteins utilized to fulfill the people nutrition (Rudini and Ayustaningwarno, 2013), carbohydrates and lipids (Astawan et al., 2014). According to the Indonesian Central Bureau of Statistics (2016), the soybean plant production in Indonesia were fluctuative since 1993 until 2015. This condition was due to the various factors as one of which was because of the plant-disturbing organisms. Pustule caused by *Xanthomonas axonopodis* pv. *glycines* (Xag) is an important disease in soybean plant (Yanti et al., 2017). On the vulnerable varieties, the soybean pustule disease can cause a reduced soybean production until 15-50% (Prathuangwong and Amnuaykit, 1987).

One of the controls that can be performed to suppress the cause of soybean pustule disease is by utilizing an antagonistic agent. Antagonistic agent can be isolated from the phyllosphere (Lindow and

Brandl, 2003), rhizosphere (Bustaman et al., 2006), rhizoplane (Cazorla et al., 2007). Phyllosphere is the leaf surface region affected by physical and chemical factors that affect the microbial development on the leaf surface. *Pseudomonas fluorescens*, *P. putida*, *P. syringae*, *Erwinia agglomerans*, Curtobacterium, *Bacillus pumilus*, *B. mycoides* are bacterial microbe types on the leaf phyllosphere (Kucheryava et al., 1999).

According to Stein (2005), *Bacillus* is an antagonistic agent used as a biocontrol due to producing peptide antibiotics, namely subtilisin, subtilosin, bacilysin, and surfactin. *B. pumilus* is capable of producing bacitracin compounds (Awais et al., 2007). *Bacillus* isolated from the corn plant phyllosphere was capable of suppressing *Exerohilum turcicum* in-vitro (Sartoni et al., 2015). The study results of Wartono et al. (2015) explained that *B. subtilis* as biocontrol agents could suppress the development of bacterial leaf blight disease caused by *X. oryzae* pv. *oryzae* bacteria (Xoo) until 21.7%.

According to Sumartini and Rahayu (2017), weeds that live among plants are the alternative hosts used by the microorganisms to sustain their life cycles. Weeds that abundantly dominate the soybean plantation are *Cynodon dactylon*, *Cyperus rotundus*, *Digitaria ciliaris*, *Eclipta alba* (Prayogo et al., 2017). Accordingly, it is necessary to perform an exploration of *Bacillus* from weed phyllosphere as an antagonistic agent against Xag. This is given as *Bacillus* and Xag are from the same habitat, namely phyllosphere, therefore it is expected that *Bacillus* obtained can suppress Xag, when applied as an antagonistic agent.

MATERIAL AND METHOD

Isolate of *Xanthomonas axonopodis* pv. *glycines*

X. axonopodis pv. *glycines* isolate used was the collection of Plant Disease Laboratory, Plant Protection, Faculty of Agriculture, University of Jember. Bacteria were cultured on the Yeast Peptone Glucose Agar (YPGA) medium with the incubation period of 48 hours at the room temperature, then confirmed through Gram, hypersensitive response (HR), and pathogenicity test (Schaad et al., 2001).

Bacillus Exploration and Isolation

Weed samples were taken from the soybean plantation in Jember Regency region, East Java, from several locations with different geographical conditions. Weed samples were taken from some points on the soybean plantation.

Isolation was performed using the method of Nurfitriani et al. (2016) and Arwiyanto (1997). The weed leaves were cut 1×1 cm and 1 g of cut leaves were moved into the 20 ml sterile water and shaken for 30 minutes. The bacterial suspension was taken 1 ml and moved into the test tube containing 9 ml sterile water. The suspension was heated at 80°C for 10 minutes, then made a serial dilution. One hundred microliters suspension on 10^5 dilutions was grown on YPGA media and incubated for 48 hours at the room temperature. The bacterial colonies grown were purified and performed Gram and hypersensitive test.

Gram test was done through on ose needle of 48-hour bacteria were put on the object glass and dropped 3% KOH, then stirred and lifted slowly (Chun & Vidaver in Schaad et al., 2001). Hypersensitive test was assayed using a bacterial suspension with the density of 10^8 cfu/ml was injected on the tobacco leaf and incubated at the room temperature for 72 hours (Chun & Vidaver in Schaad et al., 2001). The morphological characterization was performed by observing a bacterial colony, including the colony shape, color, margin, and elevation based on Capuccino and Sherman (1992).

Bacillus Screening

Bacterial screening was conducted by performing the antagonistic test using a dual platting method (Nurcahyanti et al., 2013). Bacteria were grown on the YPGA media with a sterile toothpick and incubated for 48 hours at the room temperature. Petri dish was flipped and 1 mL chloroform was dropped on the lid, then stood for 2 hours at the room temperature. The petri dish position was returned, then Xag suspension was poured onto the media surface as much as 200 µL in 4 ml 0.6% water agar medium. Then incubated for 24 hours at the room temperature and measured the inhibition zone formed by measuring the inhibition zone radius on the four colony margin sides. The inhibition mechanism test was performed by taking the agar media on the inhibition zone and moved into the test tube containing 0.5% peptone water. The bacteria growth observation was performed for five days by viewing the media opacity.

Bacillus characterization

The characterization of *Bacillus* spp. was performed by testifying their physio-biochemical character based on the characteristics and methods described by Chun & Vidaver (in Schaad et al., 2001).

Growth test at 45°C and 65°C. The 48-hour bacteria were suspensed in the sterile water and as much as 75 µl was grown on 1% liquid peptone media, then incubated at 45°C and 65°C. Opacity was observed for 5 days of incubation period. The positive reaction showed as the media turned opaque.

Growth test at pH 5.7. The 48-hour bacteria were suspended in the sterile water and as much as 75 µl was grown on 1% liquid peptone media with pH 5.7. Opacity was observed for 5 days of incubation period. The positive reaction showed as the media turned opaque.

Growth test at 7% NaCl. The 48-hour bacteria were suspensed in the sterile water and as much as 75µl was grown on 1% liquid peptone media with 7% NaCl. The bacterial growth observation was performed for 14 days.

Anaerobic growth test in glucose broth. One ose of 48-hour bacteria was grown on Hugh and Leifson's OF glucose broth media, which were put in anaerobic condition. The anaerobic condition was formed by pouring a sterile paraffin with the depth of 1 cm and incubated at 24°C. The observation was performed for 14 days against the media color alteration from blue-green to yellow.

Acid production test. The test was performed using mannitol-dextrose as the carbon source. One ose needle of 48-hour bacteria was grown on Hugh and Leifson's OF glucose broth media, which were put in anaerobic condition. The anaerobic condition was formed by pouring a sterile paraffin with the depth of 1 cm and incubated at 24°C. The observation was performed for 14 days against the media color alteration from blue-green to yellow.

Starch hydrolysis test. The 48-hour bacteria were grown on the starch medium, then incubated for 2 days at the room temperature and dropped a starch reagent.

Catalase test. The 48-hour bacteria were moved into the object glass using an ose needle, then dropped H_2O_2 and mixed slowly.

RESULT DAN DISCUSSION

Rejuvenation of *X. axonopodis* pv. *glycines*

Xag isolates showed a colony with yellow, circular, mucoid, flat margin, Gram negative, and capable of hydrolyzing starch (Sain and Gur, 2013). A positive HR test indicated that the bacteria are pathogenic and virulent, which are capable of causing leaf pustules (Figure 1).

Bacillus exploration

The isolation results from 11 weed species of several locations obtained 31 isolates survived at 80°C with Gram positive, HR negative, non-pathogenic characteristics on the soybean. These isolates were suspected as *Bacillus* (Table 1). *Bacillus* can live at an extreme temperature as forming a sustained structure to survive. Bacteria of the *Bacillus* genus can form endospore which make these species survive against physical and chemical factors (Hatmanti, 2000), namely an extreme temperature, pH, and salinity (Pratita and Putra, 2012).

The following exploration results had various morphologies and mostly showed a milky color, irregular shape, irregular margin, rough surface, and unmucoid (Table 2). The phyllospheric microbial community are varied with highly abundant and variation which is also affected by the leaf area and thickness, organic materials, region climate, as well as exudates removed by the plants (Thomson *et al.,* 1993).

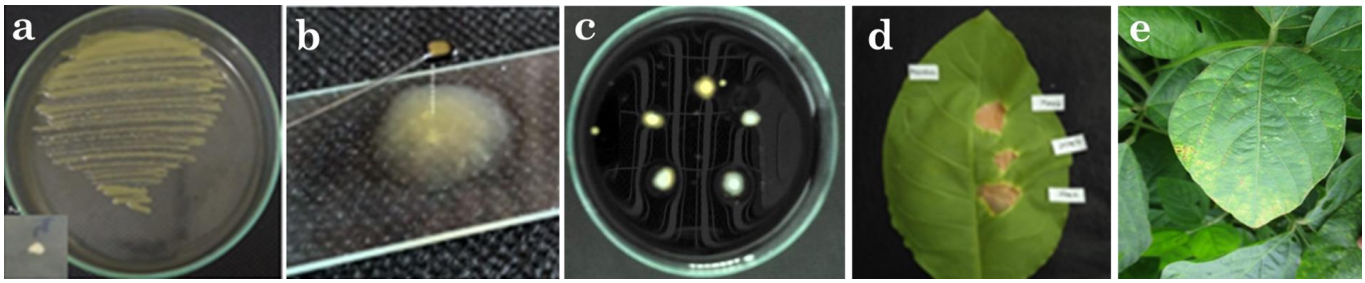


Figure 1. *X. axonopodis* pv. *glycines* bacteria, (a) *Xag* colony, (b) Gram test (Gram negative), (c) Starch hydrolysis test (positive), (d) Hypersensitive reaction and pathogenicity test (positive)

Table 1. *Bacillus* spp. isolates from weed phyllosphere

Location	Weed	Isolate Code	Gram Test	HR Test
Sukorejo, Bangsalsari -8°13'35",113°31' 47",55,0 m, 146°	*Basilicum polystachyon*	Bg a (3)	+	-
		Bg a (4)	+	-
Sukorejo, Bangsalsari -8°13'35",113°31'147", 54,0 m, 4°	*Mikania micrantha*	Bg b (4)	+	-
Petung, Bangsalsari -8°12'9", 113°34'8", 65,0 m, 218°	*Hyptis capitata*	Bg c (1)	+	-
		Bg c (3)	+	-
		Bg c (5)	+	-
Petung, Bangsalsari -8°12'9",113°34' 65",137°	*Physalis angulate*	Bg d 1(1)	+	-
		Bg d 1(2)	+	-
		Bg d 1(4)	+	-
	Brachiaria mutica	Bg d 2(1)	+	-
		Bg d 2(2)	+	-
Cangkring, Jenggawah -8,29115, 113,6672, 87, 7m	*Brachiaria mutica*	Bp 2(2)	+	-
		Bp 2(4)	+	-
Wonojati, Jenggawah -8°16'49", 113°38'18", 90,0 m, 159°	*Cynodon dactylon*	Jg 1 (1)	+	-
		Jg 1 (3)	+	-
		Jg 1(4)1	+	-
	Cleome rutidosperma	Jg 2 (1)	+	-
		Jg 2 (2)	+	-
		Jg 2 (4)	+	-
	Trianthema portulacastrum	Jg 3 (2)	+	-
		Jg 3 (6)	+	-
Krajan Barat, Jelbuk -8°4'55", 113°46'7", 247,0 m, 36°	*Mimosa pudica*	Jb 1	+	-
		Jb 3	+	-
Jubung, Sukorambi. -8°11'44", 113°38'5", 93,0 m	*Alternanthera philoxeroides*	Jb g 1 (3)	+	-
	Ipomoea aquatica	Jb g 1 (5)	+	-
		Jb g 2 (1)	+	-
		Jb g 2 (2)	+	-
	Brachiaria mutica	Jb g 2 (4)	+	-
		Jb g 3 (1)	+	-
		Jb g 3 (2)	+	-

Table 2. *Bacillus* spp. morphological characteristics

No	Isolate code	Colony Morphology			
		Color	Shape	Margin	Elevation
1	Bg a (3)	Pale white	Irregular	Irregular	Rough
2	Bg a (4)	Pale white	Irregular	Irregular	Rough
3	Bg b (4)	Pale white	Irregular	Entire	Flat
4	Bg c (1)	Pale white	Irregular	Irregular	Flat
5	Bg c (3)	Colorless	Circular	Undulate	Convex
6	Bg c (5)	Pale white	Circular	Entire	Convex
7	Bg d 1(1)	Pale white	Irregular	Irregular	Flat
8	Bg d 1(2)	Pale white	Irregular	Irregular	Flat
9	Bg d 1(4)	Pale white	Irregular	Irregular	Flat
10	Bg d 2(1)	Pale white	Irregular	Irregular	Flat
11	Bg d 2(2)	Pale white	Circular	Entire	Convex
12	Bg d 2(3)	Pale white	Circular, mucoid	Entire	Flat
13	Bp 2 (2)	Pale white	Irregular	Irregular	Flat
14	Bp 2 (4)	Pale white	Irregular	Irregular	Rough
15	Jg 1 (1)	Colorless	Circular, mucoid	Irregular	Convex

Table 2. *Bacillus* spp. morphological characteristics (*continued*)

No	Isolate code	Colony Morphology			
		Color	Shape	Margin	Elevation
16	Jg 1 (3)	Milky	Irregular	Irregular	Rough
17	Jg 1 (4)1	Pale white	Irregular	Irregular	Flat
18	Jg 2(1)	Pale white	Circular, mucoid	Entire	Flat
19	Jg 2(2)	Pale white	Irregular	Irregular	Rough
20	Jg 2(4)	Pale white	Irregular	Irregular	Rough
21	Jg 3 (2)	Pale white	Irregular, mucoid	Irregular	Flat
22	Jg 3 (6)	Pale white	Irregular	Irregular	Rough
23	Jb (1)	Pale white	Irregular	Entire	Rough
24	Jb (3)	Pale white	Irregular	Irregular	Flat
25	Jbg 1 (3)	Colorless	Circular, mucoid	Entire	Convex
26	Jbg 1 (5)	Pale white	Irregular	Irregular	Flat
26	Jbg 2 (1)	Pale white	Irregular, mucoid	Irregular	Convex
28	Jbg 2 (2)	Pale white	Irregular	Irregular	Flat
29	Jbg 2 (4)	Colorless	Circular, mucoid	Entire	Flat
30	Jbg 3 (1)	Pale white	Irregular	Irregular	Flat
31	Jbg 3 (2)	Colorless	Irregular	Undulate	Convex

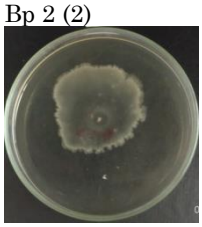
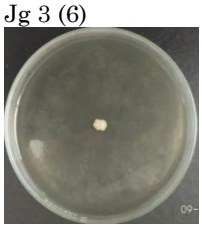
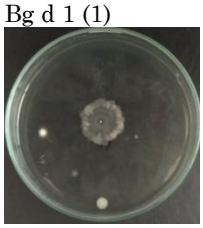
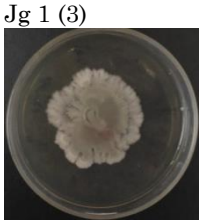
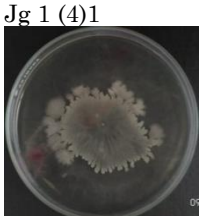
Table 3. *Bacillus* spp. capabilities in inhibiting *Xag*

No.	Isolate code	Inhibitory zone against *Xag* (mm)	No.	Isolate code	Inhibitory zone against *Xag* (mm)
1	Bg a (3)	3	17	Jg 1 (4) 1	8.5
2	Bg a (4)	0	18	Jg 2 (1)	0
3	Bg b (4)	4	19	Jg 2 (2)	0
4	Bg c (1)	6	20	Jg 2 (4)	0
5	Bg c (3)	0	21	Jg 3 (2)	7
6	Bg c (5)	0	22	Jg 3 (6)	11
7	Bg d 1 (1)	10	23	Jb (1)	5
8	Bg d 1 (2)	3	24	Jb (3)	3.5
9	Bg d 1 (4)	2	25	Jbg 1 (3)	6
10	Bg d 2 (1)	4	26	Jbg 1 (5)	7
11	Bg d 2 (2)	2	27	Jbg 2 (1)	2.5
12	Bg d 2 (3)	0	28	Jbg 2 (2)	0
13	Bp 2 (2)	15	29	Jbg 2 (4)	7.7
14	Bp 2 (4)	8	30	Jbg 3 (1)	4
15	Jg 1 (1)	0	31	Jbg 3 (2)	7
16	Jg 1 (3)	9.5			

***Bacillus* spp. screening**

The inhibitory effect test results of *Bacillus* spp. against Xag obtained 22 isolates were capable of inhibiting Xag pathogen and 9 other isolates did not show an inhibition (Table 3). This capability was shown from the occurrence of clear zone formed around Bacillus spp. colony. 22 isolates showed varied clear zones from 2 until 15 mm (Table 3). The study result of Marcic et al. (2018) presented that *Bacillus* spp. had various abilities to inhibit *X. vesicatoria* and *Clavibacter michiganensis* subsp. *michiganensis*. The inhibitory mechanism of all *Bacillus* spp. against Xag were bacteriostatic, which means that Bacillus spp. are inhibiting and not lethal. This was suspected as *Bacillus* spp. produced certain compounds and Xag bacteria were sensitive against the compounds, therefore disrupting the metabolism process in the bacterial cell and causing an inhibited bacterial growth.

Table 4. The characteristics of five *Bacillus* spp. strain as potential antagonistic agents against Xag

Bacterial colony (48 hours)	Characteristic	Isolate origin	Colony diameter (mm)	Inhibitory zone (mm)	Inhibitory mechanism
Bp 2 (2) 	Pale white color, rough surface, irregular shape, irregular margin, flat elevation.	*Brachiaria mutica*	45	15	Bacteriostatic
Jg 3 (6) 	Pale white color, rough surface, irregular shape, irregular margin, flat elevation.	*Trianthema portulacastrum*	9	11	Bacteriostatic
Bg d 1 (1) 	Pale white color, rough surface, irregular shape, irregular margin, flat elevation.	*Physalis angulata*	25	10	Bacteriostatic
Jg 1 (3) 	milky color, rough surface, irregular shape, irregular margin, flat elevation.	*Cynodon dactylon*	47	9.5	Bacteriostatic
Jg 1 (4)1 	Pale white color, rough surface, irregular shape, irregular margin, flat elevation.	*Cynodon dactylon*	68	8.5	Bacteriostatic

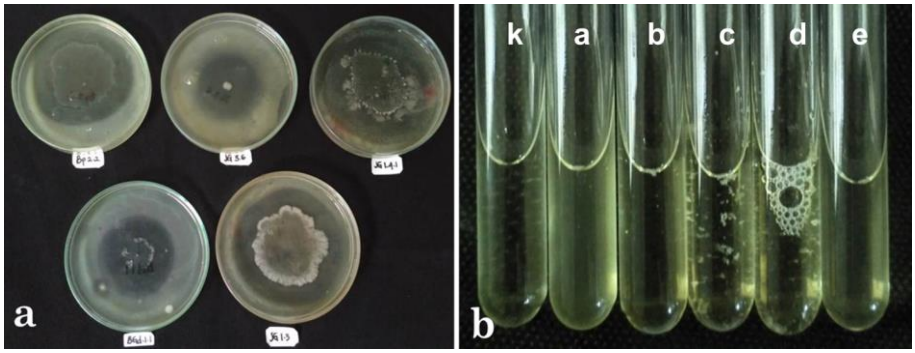


Figure 2. The *Bacillus* spp. isolate inhibition against *Xag*. Note: (a) inhibitory zone formation, (b) five *Bacillus* spp. isolates inhibitory mechanism *:* k) control a) Bp2(2), b) Jg3(6), c) Jg1(4), d) Bg d1(1), e) Jg1(3)

Table 5. The physio-biochemical characteristics of five *Bacillus* spp. isolates

Test	Isolate code					Chun & Vidaver in Schaad *et al.,* (2001)						
	Bp 2(2)	Bg d 1(1)	Jg 1(3)	Jg 3(6)	Jg 1(4)1	*licheniformis*	*subtilis*	*coagulan*	*alvei*	*brevis*	*lateros-porus*	*mace-rans*
Gram	+	+	+	+	+	+	+	+	I	I	I	I
Hypersensitive	-	-	-	-	-	-	-	-	-	-	-	-
Growth on the liquid media:												
- 45°C	+	+	+	+	+	+	+	+	V	V	V	V
- 65°C	-	+*	+	-	+	NT	NT	NT	NT	NT	NT	NT
- pH 5.7	+	+	+	+	+*	+	+	+	+	V	+	+
- NaCl 7%	-	+	+	+	+	+	+	-	-	-	-	-
Anaerobic growth on glucose broth	+*	+	+*	+*	+	+	-	NT	+	-	+	+
Acid production:												
- mannitol	-	+	+	+	+	+	+	V	-	V	+	+
- dextrose	-	-	-	+	-	NT	NT	NT	NT	NT	NT	NT
Starch hydrolysis	+	+	+	+	+	+	+	+	+	-	-	0
Catalase	+	+	+	+	+	NT	NT	NT	NT	NT	NT	NT

Table 6. The living capability of five *Bacillus* spp. on the environmental condition and carbon source

Isolate code	45°C	65°C	NaCl 7%	pH 5,7	Anaerobic growth in glucose broth	Mannitol	Dextrose
control	-	-	-	-	-	-	-
Jg 1(3)	+++	+	+++	++	+	+	-
Jg 3(6)	+++	-	++++	+	+	+	+
Jg 1(4)1	++	+	+++	++	++	++	-
Bg d 1(1)	++	+	+++	++	++	+	-
Bp 2(2)	++	-	-	+	+	+	-

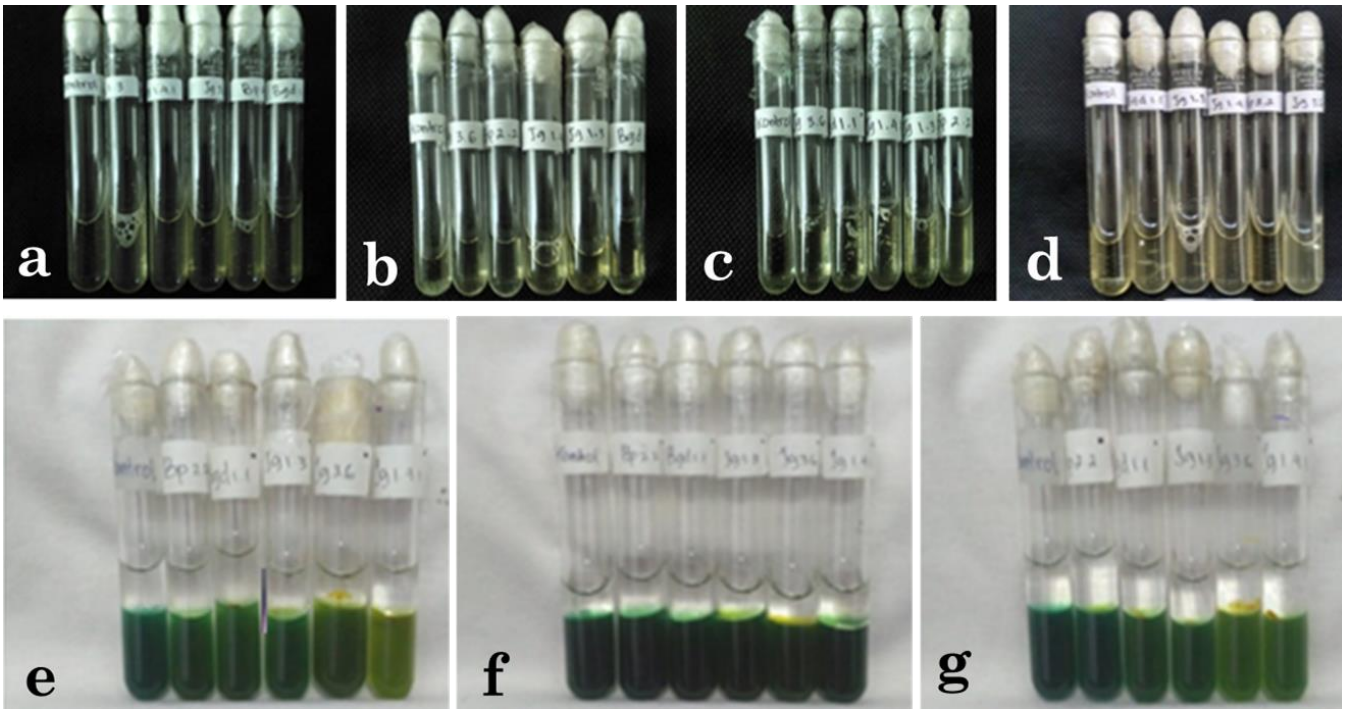


Figure 3. The living capability of five *Bacillus* spp. (a) growth at 45°C, (b) growth at 65°C, (c) pH 5,7; (d) NaCl 7%, (e) glucose carbon source utilization, (f) dextrose carbon source utilization, (g) mannitol carbon source utilization.

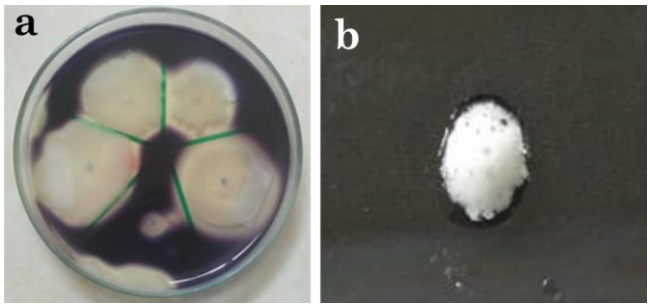


Figure 4. *Bacillus* spp. test, (a) Starch hydrolysis (positive), (b) Catalase (positive)

According to Soesanto (2013), Bacillus can play the role as plant pathogen biocontrol agent through the antibiotic mechanism by producing the antimicrobial compounds, namely antibiotics, peptides, phenolic compounds, enzymes, alkaloids, and siderophore. *B. subtilis* is capable of producing antibiotics in the form of bacitracin and subtilin. According to Javandira et al. (2013), those antibiotic compounds will inhibit the protein synthesis on bacteria, therefore inhibiting the growth.

Based on the capabilities of *Bacillus* spp. in inhibiting Xag, five potential isolates were chosen as antagonistic agents based on the diameter of inhibition zone. These five isolates were Bp 2(2), Jg 3(6), Bg d 1(1), Jg 1(3) and Jg 1(4)1 with various characteristics (Table 4, Figure 2). The Jg1(4)1 isolate showed the largest colony diameter with 68 mm, while the largest inhibition zone against Xag was obtained from Bp2(2) isolate. The growth of Jg1(4)1 isolate as the largest colony shows a shorter generation period, therefore the bacterial population increases in faster period. The Bp2(2)

isolate had the largest inhibitory effect was suspected as producing high content of inhibitory compounds or more various compound types, therefore the inhibitory capability was larger than other isolates. As an antagonistic agent, *Bacillus* which has faster growth capability play the role on inhibition mechanism in the form of competitions, either growth space or nutrient. Bacillus with large inhibitory effect will be able to suppress the metabolism process better, therefore the pathogenic bacterial will be more inhibited. On the inhibitory mechanism test, it showed that Jg1(4) 1 and Bg d1(1) isolate formed an aggregate against Xag growth, while other isolates grew spreading on the liquid media.

The physio-biochemical test results (Table 5) showed that *Bacillus* strain Jg 3(6), Bg d 1(1), Jg 1(3), Jg 1(4)1 had the similar characteristics to *B. licheniformis* and Bp 2(2) was similar to *B. coagulans*.

Five *Bacillus* sp. isolates had some similarities in the physio-biochemical tests, however having different growth capabilities on the temperature, NaCl content, media acidity, and carbon source (Table 6, Figure 3). Each isolate has its own advantages to survive on different environmental condition and capability of utilizing the carbon source. This diversity is suspected to cause *Bacillus* spp. can grow together as a phyllospheric community.

Bacillus was capable of hydrolyzing the starch marked by the occurrence of the clear zone around the bacterial colonies after given some drops of iodine solution (Figure 4). The clear zone around the bacterial colonies indicated that starch in the media had been hydrolyzed by amylase enzyme produced by the bacteria to become simplified sugar compounds. According to Amri et al. (2010), the bacterial capability of producing amylase is determined by the structural gene existence or absence, which regulates the amylase protein synthesis inside the bacterial cell. Catalase test results performed showed positive results. This was marked by the occurrence of gas bubbles on each *Bacillus* isolate dropped with H_2O_2. According to Suhartanti et al. (2010), the occurrence of catalase activity can be known from the O_2 gas bubbles formed. Hydrogen peroxide (H_2O_2) can be decomposed by catalase enzyme produced by the microorganisms to become $H_2O + O_2$.

The exploration results obtained five *Bacillus* spp. strains that had the largest inhibitory effect against Xag, namely strain Bp 2(2), Jg3(3), Bg d1(1), Jg 1(3) and Jg 1(4)1. Strain Bp 2(2) had the largest inhibition zone with 15 mm and Jg 1(4)1 showed the fastest colony growth with 68 mm. The physio-biochemical test results showed that Bacillus strain Jg 3(6), Bg d 1(1), Jg 1(3),Jg 1(4)1 had similar characteristics to *B. licheniformis* and Bp 2(2) was similar to *B. coagulans*.

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REFERENCES

Amri E, N Widhyastuti, IM Artika. 2010. Aktivitas amilase bakteri yang diisolasi dari sumber air panas Ciseeng Bogor. Saintek 2(1): 23-33.

Arwiyanto T. 1997. Pengendalian hayati penyakit layu penyakit tembakau: 1. Isolasi bakteri antagonis. Perlindungan Tanaman Indonesia 3(1): 54-60.

Astawan M, NR Adiningsih, NS Palupi. 2014. Evaluasi kualitas nuget dari berbagai varietas kedelai. Pangan 23(3): 244-255.

Awais M, AA Shah, A Hamed, F Hasan. 2007. Isolation, Identification and optimization of bacitracin produced by *Bacillus* sp. Pak. J. Bot. 39(4): 1303-1312.

Badan Pusat Statistika. 2016. Produksi Kedelai Menurut Kabupaten Kota di Jawa Timur Tahun 2007-2016. Jawa Timur: Badan Pusat Statistik, Indonesia.

Bustamam H. 2006. Seleksi mikroba rizosfer antagonis terhadap bakteri *Ralstonia solanacearum* penyebab penyakit layu bakteri pada tanaman jahe di lahan tertindas. Jurnal Ilmu-ilmu Pertanian Indonesia 8(1): 12-18.

Cappucino JG, N Sherman. 1992. Microbiology a Laboratory Manual 3rd Edition. Calif Redwood City, California: The Benjamin Cummings Publishing Company Inc.

Cazorla, FM, D Romero, AP Garzia, BJJ Lugtenberg, A de Vicente, G Bloemberg. 2007. Isolation and characterization of antagonistic *Bacillus subtilis* strains from the avocado rhizoplane displaying biocontrol activity. Appl. Microbiol. 103(1): 1950-1959.

Hatmanti A. 2000. Pengenalan *Bacillus* sp. Oseana 25(1): 31-41.

Javandira C, LQ Aini, AL Abadi. 2013. Pengendalian penyakit busuk lunak umbi kentang (*Erwinia carotovora*) dengan memanfaatkan agens hayati *Bacillus subtilis* dan *Pseudomonas fluorescens*. Jurnal Hama dan Penyakit Tumbuhan 1(1): 90-98.

Kucheryava N, M Fiss, G Auling, RM Kropenstedt. 1999. Isolation and characterization of epiphytic bacteria

from the phyllosphere of apple, antagonistic in vitro to *Venturia inaequalis*, the causal agent of apple scab. Syst. Appl. Microbiol. 22(1): 472-478.

Lindow SE, MT Brandl. 2003. Microbiology of the phyllosphere. Appl. Environ. Microbiol. 69(4): 1875-1883.

Marcic ST, V Todorovic, O Stanojevic, T Beric, S Stankovic, B Todorovic, I Potocnik. 2018. Antagonistic potential of *Bacillus* spp. Isolates againts bacterial pathogens of tomato and fungal pathogen of pepper. Pestic. Phytomed. 33(1): 9-18.

Nurcahyanti SD, T Ariwiyanto, D Indradewa, J Widada. 2013. Isolasi dan seleksi pseudomonad flourescens pada risosfer penyambungan tomat. Berkala Ilmiah Pertanian. 1(1): 15-18.

Nurfitriani R, NPRA Krishanti, A Akghdiya, AT Wahyudi. 2016. Penapisan bakteri filosfer penghasil senyawa bioaktif anti *Xanthomonas oryzae* pv. *oryzae* penyebab penyakit hawar daun bakteri pada padi. Sumberdaya Hayati 2(1): 19-25.

Prathuangwong S, K Amnuaykit. 1987. Studies on tolerance and rate-reducing bacterial pustule of soybean cultivars lines. Kasetsart Journal Natural Science 21(4): 408-426.

Pratita MYE, SR Putra. 2012. isolasi dan identifikasi bakteri termofilik dari sumber mata air panas di songgoriti setelah dua hari inkubasi. Teknik Pomits 1(1): 1-5.

Prayogo DP, HT Sebayang, A Nugroho. 2017. Pengaruh pengendalian gulma pada pertumbuhan dan hasil tanaman kedelai (*Glycine max* (L.) Merril) pada berbagai sistem olah tanah. Produksi Tanaman 5(1): 24-32.

Rudini B, F Ayustaningwarno. 2013. Kadar protein, serat, triptofan, dan mutu organoleptik kudapan ekstrusi jagung dengan substitusi kedelai. Nutrition Collage 2(3): 373-381.

Sartoni M, A Nesci, A Formento, M Etcheverry. 2015. Selection of potential biological control of *Exserohilum turcium* with epiphytic microorganism from maize. Revista Argentina de Microbiología 47 (1): 62-71.

Sain SK, HN Gour. 2013. Pathological and physio-biochemical characterization of *Xanthomonas axonopodis* pv. *glycines*, incitent of *Glycine max* leaf pustules. Indian Phytopathology 66(1): 20-27

Schaad NW, JB Jones, W Chun. 2001. Laboratory Guide for Identification of Plant Pathogenic Bacteria. USA: Onacid.

Soesanto L. 2013. Pengantar Pengendalian Hayati Penyakit Tanaman. Depok: PT RajagrafindoPersada.

Stein T. 2005. *Bacillus subtilis* antibiotics: structures, syntheses and specific function. Mol. Microbiol. 56(4): 845-857.

Suhartanti M, PR Sarjono, ALN Aminin. 2010. Studi filogeni dan uji potensi enzim ekstraseluler (amilase, ß-galaktosidase, protease, katalase) Isolat *Alicyclobacillus* sp. Gedong Songo. Kimia Sains dan Aplikasi 13(3): 13-3.

Sumartini, M Rahayu. 2017. Penyakit embun tepung dan cara pengendaliannya pada tanaman kedelai dan kacang hijau. Jurnal Penelitian dan Pengembangan Pertanian 36(2): 59-66.

Thomson JP, MJ Bailey, JS Fenlon, TR Fermor, AK Lilley, JM Lynch, PJ McCormack, MP McQuilken, KJ Purdy, PB Rainey, JM Whipps. 1993. Quantitative and qualitative seasonal changes in the microbial community from the phyllosphere of sugar beet (*Beta vulgaris*). Plant and Soil 150(2): 177-191.

Wartono, Giyanto, KH Mutaqin. 2015. Efektivitas formulasi spora *Bacillus subtilis* B12 sebagai agen pengendali hayati penyakit hawar daun bakteri pada tanaman padi. Jurnal Penelitian Pertanian Tanaman Pangan 34(1): 21-28.

Yanti Y, T Habazar, Z Resti. 2017. Formulasi padat rhizobakteria indigenus *Bacillus thuringiensis* TS dan waktu penyimpanan untuk mengendalikan penyakit pustul bakteri *Xanthomonas axonopodis* pv. glycines. Jurnal Hama dan Penyakit Tumbuhan Tropika 17(1): 9-18.