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Diversity, Functional Analysis and Antagonistic potential of Culturable-Dependent Soil Bacteria from Rhizosphere Area of *Fusarium oxysporum*-infected Banana Trees.

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Abstract

This study aimed to analysis diversity of culturable bacteria that isolated from rhizosphere area of banana trees. Soil samples were collected from rhizosphere areas of banana tree which showed infected and non-infected by *Fusarium oxysporum* f.sp. cubense (Foc). Four areas were chosen with two soil samples representing Foc-infected soils and two others for non-infected soils. Soil bacteria were grown on NA agar media with serial dilution 1-10^6 using pour plate method. Different bacterial colonies were isolated and distinguished according morphology, colour and microbiology characters. Twenty-six different colonies were isolated with ten colonies from non-infected soils and 16 colonies from Foc-infected soils. Functional analysis showed 70% isolates (18 isolates) were atmospheric-N2 fixation bacteria. All colonies are IAA producer with various level but none of the isolates categorized as phosphate solubilizing bacteria. Molecular identification using 16S rRNA gene showed 88,5% isolates were *Bacillus* which consisted of 17 different species. 12,5% of the rest were *Enterobacter tabaci, Lysinibacillus xylanilyticus* and *Staphylococcus arlettae*. Antagonistic test with Foc showed 13 strain of *Bacillus* and 2 other bacteria (*Lysinibacillus xylanilyticus* and *Staphylococcus arlettae)* have potential to control developing of Foc. The best inhibition was showed by *B. cereus* CCM 2010.

Keyword: Inhibition, *Fusarium oxysporum* f.sp.cubense, Rhizosphere bacteria

Introduction

Banana is one of the most popular fruits consumed worldwide. Indeed, some areas in Africa, South Asia, and Tropical America consume banana as a staple food (Karamura et al, 2012; Butler, 2013) or as a significant addition of diets (Perrier et al, 2011). Economically trade of banana has significant value in fruit global production. Many countries put banana as an important agricultural commodity with a significant impact on foreign change such as in Ecuador, Costa Rica, Panama, Columbia, Guatemala, Philippines, Honduras, Cameroon and some other countries in South Asia (FAOSTAT 2015). In 2016, global banana production was aimed for exporting was valued at USD 8 billion and thus it placed Banana as the most popular fruit in industrialized countries (FAO, 2018).

Despite its value and importance contribution, worldwide banana production faces decreasing in production. Most of the banana is cultivated and multiplied by vegetative propagation which is vulnerable to infection by various diseases. One of the most devastating disease of Banana is Fusarium wilt or Panama wilt disease which caused by soil-borne fungi *Fusarium oxysporum* f.sp. cubense (foc) (Ploetz, 2005). *Fusarium oxysporum* f.sp. cubense race 1 has been identified as the main caused of 'Gross Michael' total destruction in the 1890s (Ploetz, 2005). The emerging of new banana cultivar, Cavendish, then replaced the world banana production since Cavendish was resisted Fusarium wilt cultivar (Stover, 1962). However, in the 1960s, it had reported a new race of Foc tropical race 4 (TR4) able to infected Cavendish cultivar in Taiwan and spread rapidly from Southeast Asia to the whole world (Ploetz, 1994; Butler 2013; Garcia et al. 2013; Ordonez et al. 2015; Ploetz et al. 2015). The rapid expansion of the Panama disease epidemic is allegedly causing by massive monoculture of susceptible Cavendish bananas (Ordonez et al, 2015; Maryani et al, 2019).

Fusarium oxysporum f.sp. cubense is a soil-borne fungus that enable to survive inhospitable by producing chlamydospores. The chlamydospores were suggested persistent in decayed banana tissue or in the soil were responsible for its durability in infested soil (Stover 1962). Their long-survival in the soil is about twenty years (Stover 1962) or even longer about 40 years (Buddenhagen 2009), causing the soils of infected plantation could not be produced. Currently, there is no effective method for controlling of Foc spreading that was reported (Getha et al, 2002; Wang et al, 2013). Management of Fusarium wilt disease mainly involves application of chemical, rotation, and selection of resistant banana varieties. Although none of these methods have been reported resulted decreasing of Fusarium wilt disease since the continuous spread among continents, countries and regions are remain happen (Getha et al, 2002; Wang et al, 2013; Dale et al, 2017). Another proposed method that is accepted as risk management of Fusarium wilt outbreak is eradication (Dita et al, 2010) and quarantine the infected plantation. However, these methods were performed base on long-term suppression-containment, it may have a significant socio-economic impact (Dita et al, 2010) particularly for small farmers.

In case *of Fusarium oxysporum*, application of fungicides is main method for fungal disease control. But their potential undesirable and unpredictable environmental side effects make fungicides are not more first choice for fungal disease control (Lo, 2010). Biocontrol is considered as a safe, environmentally friendly and cost-effective method for disease control. Application of bacterial consortium for inducing suppressiveness of conducive soil against

soil-borne pathogens, especially *Fusarium oxysporum*, have been reported in several studies (Lemanceau et al, 2006; De Souza et al, 2003; Klein et al, 2011). Mechanism of disease suppressive soil is mainly drive by competition, parasitism, antibiosis, and level of microbial diversity in the soil (Kloepper et al., 1980; Mazzola and Gu, 2002; Garbeva et al, 2006; Alabouvette et al., 2009) and by soil organic matter (Shen et al, 2018). In this study, isolation and characterization of rhizosphere bacteria from banana plantation was performed for identifying potential bacteria that are able to control fusarium disease in Banana plantation.

Material and Methods

Soil sample collection

Soil samples were taken from Banana plantation of PT Perkebunan Nusantara VIII in Parakansalak, Sukabumi West Java. The soil samples were collected from rhizosphere area of banana trees which indicated infected by Foc and from healthy banana trees. From these soil conditions, two soil samples were collected from three locations for each soil conditions. About 500 gr soils were taken from 20 cm soil depth from each location and mixed homogenously. About 100 gr soils from both mixture soil conditions were kept, labelled (BH and BI) and stored in ice cooled box until they were used. This procedure was repeated twice. For abiotic data, one kg soil from each soil conditions were collected and analysed for soil physical and chemical data.

Isolation and Characterization of Bacteria isolates

The soil samples were diluted serially 10-10^{-6} and cultured in NA medium. Different bacterial colonies were isolated and distinguished according morphology, colour and microbiology characters. Selected bacteria were purified until single purified colonies for each bacterial isolate were obtained. Each selected isolate was tested their functional characters for Nitrogen-free fixed, HCN production, and IAA production.

Molecular Characterization

Total DNA of selected isolates were extracted according Mahuku (2014). PCR reaction of each selected isolate was performed by amplifying of 16S rRNA gene using the following 50 ul PCR mix containing 1 µl primer forward, 1 µl primer reverse, 25 µl MyTaq™ Red Mix, 22 µl free-nuclease water, and 2 µl DNA template. The forward primer 27F 5’ – AGAGTTTGATCMTGGCTCAG– 3’ and reverse primer 1492R 5’ –

TACGGYTACCTTGTTACGACTT – 3'. PCR was performed according set-up reaction: one minute of 95°C pre-denaturation, 35 cycles containing 15 second 95°C denaturation, 15 second of 57°C for annealing, 10 second of 72°C for extension, and 5 minutes of 72°C for final-extension. PCR products were visualized by electrophorized in 1% gel agarose and the corrected amplification band (1465 base pair) were isolated from gel agarose and sequenced.

Bioinformatic analysis

Sequencing results were then analysed by aligning with sequences with NCBI database using BLAST searching engine. Only similarity more than 95% with database was considerate as match alignment. Phylogenetic analysis was performed by multiple aligning of all identified sequences using MUSCLE - MUltiple Sequence Comparison by Log-Expectation software (https://www.ebi.ac.uk/Tools/msa/muscle/). Before multiple alignment was performed, the sequences were trimmed using GeneDoc 2.7 (https://genedoc.software.informer.com/) and the result was used for designing phylogenetic tree.

Analysis of Antagonistic Against Foc

All identified bacteria were tested for pathogenic potential by growing in Blood agar media according Buxton (2015). Only non-pathogenic bacteria were further tested for determining antagonist ability against Foc. The antagonistic test was performed using dual culture method (Ghai et al, 2017). PDA agar with five days mycelia was place at the central of TSA media. Respective non-pathogenic bacteria (24 h old) was inoculated at 2 cm juxtaposed to the Foc on each plate. TSA plate without inoculation of Foc was served as control plate. All plates were incubated at 28°C for 5 days. Antagonistic level was measured according formula: C-T/C x 100, where C is the colony growth of Foc in control, and T is the colony growth of Foc in dual culture plates. Measurement of growth diameter was done using ImageJ software (Schneider et al, 2012).

RESULT

Abiotic data and Physiological characters of isolated bacteria

Abiotic data of both soil condition (BH and BS) indicated almost similar physically and chemically of these soils. Only P_2O_5 concentration and K_2O were significantly lower in BH in comparison with BS. Both soils were also categorised as clay type since clay dominate structure of soil composition. Detail of abiotic data is presented in table 1.

Table 1. Physical and chemical data of soil samples

Sample	C-Org (%)	N-Total (%)	P_2O_5 (mg/100g)	K_2O (mg/100g)	P_2O_5 bray* (ppm)	KTK ($cmol^{(+)}$/kg)	pH	Humidity (%)	Texture		
									Sand (%)	Silt (%)	Clay(%)
Health soil (BH)	1,97	0,23	89,25	20,17	20,99	5,34	3.3	75,80	23,57	15,79	60,64
Foc-Infected soil (BS)	2.22	0.36	150.79	197.51	23.68	4.53	3.5	74.60	8.80	32.89	58.31

BH From health soil (BH) and Foc-infected soil (BS) samples, we enable isolated 10 and 16 distinctly bacterial isolate respectively. Total plate count indicated that number of bacterial colonies in BH samples (in average 6,75 x 10^5 CFU/g) were larger than in BS samples (in average 2,6 x 10^5 CFU/g). Physiological function analysis which consisted of roles for N_2-free fixation, HCN production and IAA production showed various level of these function between bacterial isolates (Table 2).

Table 2. Physiological function measurement test of bacterial isolates

No.	Isolate code	N2 fixation	HCN production	IAA production (ppm)
1	BH 14A	+	+	34,75
2	BH 14C	+	+	89,3
3	BH 15A	+	−	86,27
4	BH 15C	+	+	21,12
5	BH 16B	+	−	22,63
6	BH 16C	+	+	55,97
7	BH 24A	+	+	34,76
8	BH 34A	+	−	15,06
9	BH 34C	−	−	19,60
10	BH 34D	−	+	18,09
11	BS 14A	−	−	25,67
12	BS 14B	+	−	25,67
13	BS 15B	+	−	54,45
14	BS 24B	+	+	24,15
15	BS 24C	−	−	25,67
16	BS 24D	+	+	21,12
17	BS 24E	+	−	27,18
18	BS 24F	+	−	25,67
19	BS 25B	+	−	27,18
20	BS 25E	+	+	22,64
21	BS 26A	+	+	28,70
22	BS 26B	+	−	22,64
23	BS 34C	−	−	98,39
24	BS 35A	−	+	28,70
25	BS 35B	−	−	22,64
26	BS 35C	−	+	31,73

Molecular identification and phylogenetic tree

Molecular analysis of 16S rRNA gene of each isolated bacteria indicated *Bacillus* genus dominated identified bacteria in both soil samples. Alignment of these sequences with NCBI database using BLAST-N showed all of sequences match with NCBI database with similarity more than 98% and E-value 0.00. About 88,5% isolates were *Bacillus* which consisted of 17 different species and 12,5% of the rest were *Enterobacter tabaci, Lysinibacillus xylanilyticus* and *Staphylococcus xylaniltycus*. Detail of alignment result is presented in table 3.

Phylogenetic tree was constructed using Neighbour-joining tree without distance corrections. Three different strains of *Thermococcus litoralis* (*T. litoralis* strain JCM 8560, *T. litoralis* strain DSM 5473T, and *T. litoralis* strain DSM 5474) were used as outgroup for generating the phylogenetic tree (Figure 1). The phylogenetic tree showed clear clustering of similar species bacteria into the same group (group of *B. cereus* and group of *B. thuringiensis*)

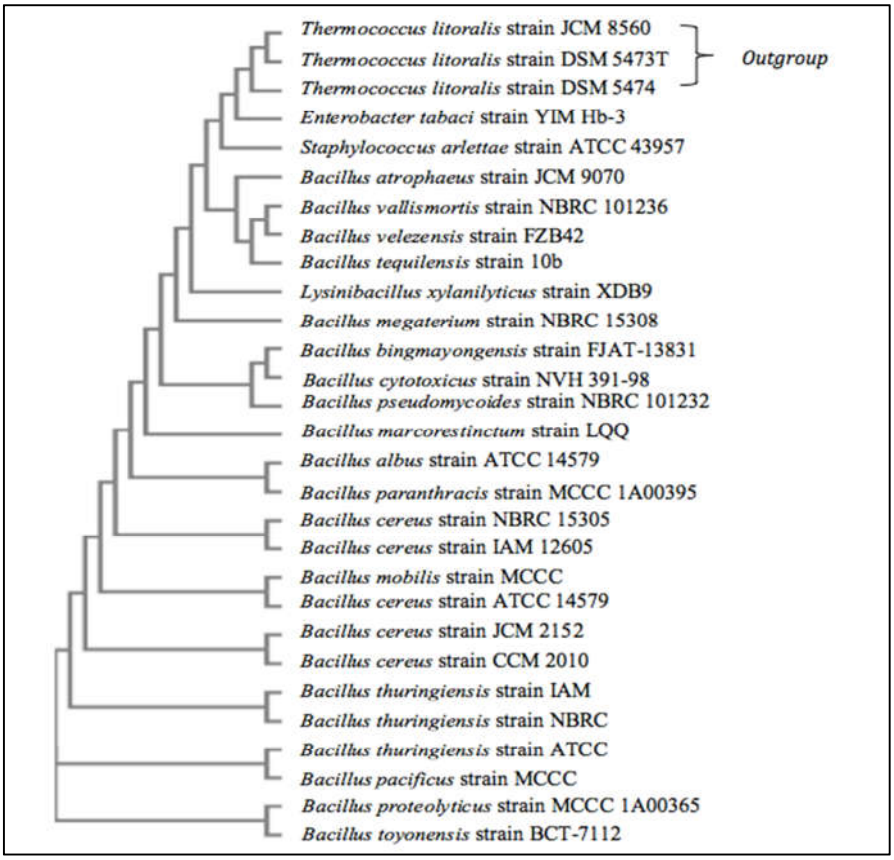

Figure 1. Phylogenetic tree of 26 identified bacteria from health and Foc-infected soil samples.

Tabel 3. Bacterial ID according alignment of 16S rRNA gene to NCBI database using BLAST-N

	ISOLATE	BACTERIAL ID	MAX SCORE	QUERY COVER	E-VALUE	IDENT	ACC NO
1	BH 14A	*Bacillus cereus* strain NBRC 15305	1875	99%	0.0	100.00%	NR_112630.1
2	BH 14C	*Bacillus cereus* strain IAM 12605	1581	100%	0.0	99.77%	NR_115526.1
3	BH 15A	*Enterobacter tabaci* strain YIM Hb-3	1797	100%	0.0	98.91%	NR_146667.2
4	BH 15C	*Bacillus velezensis* strain FZB42	1574	100%	0.0	99.20%	NR_075005.2
5	BH 16B	*Bacillus atrophaeus* strain JCM 9070	1537	100%	0.0	98.19%	NR_024689.1
6	BH 16C	*Bacillus albus* strain MCCC 1A02146	1615	100%	0.0	98.37%	NR_157729.1
7	BH 24A	*Bacillus cereus* ATCC 14579	1869	99%	0.0	100.00%	NR_074540.1
8	BH 34A	*Bacillus marcorestinctum* strain LQQ	926	100%	0.0	99.41%	NR_117414.1
9	BH 34C	*Bacillus paranthracis* strain MCCC 1A00395	1506	100%	0.0	98.93%	NR_157728.1
10	BH 34D	*Bacillus mobilis* strain MCCC 1A05942	1866	100%	0.0	99.90%	NR_157731.1
11	BS 14A	*Bacillus tequilensis* strain 10b	1954	100%	0.0	99.91%	NR_104919.1
12	BS 14B	*Bacillus cereus* strain JCM 2152	1766	100%	0.0	100.00%	NR_113266.1
13	BS 15B	*Bacillus megaterium* NBRC 15308	1659	100%	0.0	99.89%	NR_112636.1
14	BS 24B	*Bacillus proteolyticus* strain MCCC 1A00365	1639	99%	0.0	99.89%	NR_157735.1
15	BS 24C	*Bacillus cereus* strain CCM 2010	1858	99%	0.0	99.70%	NR_115714.1
16	BS 24D	*Bacillus thuringiensis* strain IAM 12077	1552	100%	0.0	99.88%	NR_043403.1
17	BS 24E	*Bacillus pacificus* strain MCCC 1A06182	1701	99%	0.0	99.68%	NR_157733.1
18	BS 24F	*Bacillus thuringiensis* strain ATCC 10792	1594	100%	0.0	99.88%	NR_114581.1
19	BS 25B	*Bacillus vallismortis* strain NBRC 101236	1517	100%	0.0	98.82%	NR_113994.1
20	BS 25E	*Bacillus bingmayongensis* strain FJAT-13831	1424	99%	0.0	96.52%	NR_148248.1
21	BS 26A	*Bacillus thuringiensis* strain NBRC 101235	1733	100%	0.0	99.89%	NR_112780.1
22	BS 26B	*Bacillus toyonensis* strain BCT-7112	1827	100%	0.0	99.90%	NR_121761.1
23	BS 34C	*Lysinibacillus xylanilyticus* strain XDB9	1701	99%	0.0	99.05%	NR_116698.1
24	BS 35A	*Staphylococcus arlettae* strain ATCC 43957	1591	99%	0.0	99.10%	NR_024664.1
25	BS 35B	*Bacillus pseudomycoides* strain NBRC 101232	1642	100%	0.0	99.56%	NR_113991.1
26	BS 35C	*Bacillus cytotoxicus* strain NVH 391-98	1528	100%	0.0	98.39%	NR_074914.1

Potency against Foc

Pathogenetic test the 26 identified bacteria resulted six bacteria which categorized as gamma (γ) haemolytic bacteria, whereas the rest bacteria were categorized as alpha haemolytic bacteria and beta haemolytic bacteria were 15 and 13 bacteria respectively (Figure 2). The Beta haemolytic bacteria have excluded from antagonistic test against Foc, due to their pathogenic property. The Gamma and Alpha haemolytic bacteria were then proceeded further for antagonistic test. The antagonistic test was performing to indicating the potential of these bacteria for inhibiting Foc. Data showed out of 25 bacteria, 15 bacteria could inhibit Foc growth with various level of inhibition. Two bacteria are belonging Gamma haemolytic bacteria and 13 bacteria are Alpha haemolytic bacteria. Detail of inhibition level shows in table 4.

Table 4. Inhibition rate of isolated alpha and gamma haemolytic bacteria against Foc in in vitro dual culture assay

No	Bacteria ID	Type of hemolytic	Origin of sample	Inhibition rate (%)
1.	*Bacillus cereus* CCM 2010	Alpha	BS	25.68
2.	*Lysinibacillus xylanilyticus* XDB9	Alpha	BS	24.67
3.	*Bacillus thuringiensis* NBRC 101235	Alpha	BS	22.68
4.	*Staphylococcus arlettae* ATCC 43957	Alpha	BS	22.30
5.	*Bacillus cytotoxicus* NVH 391-98	Alpha	BS	21.52
6.	*Bacillus pseudomycoides* NBRC 101235	Alpha	BS	21.26
7.	*Bacillus mobilis* MCCC IA05942	Alpha	BH	19.74
8.	*Bacillus toyonensis* BCT-7112	Alpha	BS	17.46
9.	*Bacillus thuringiensis* IAM 12077	Alpha	BS	12.04
10.	*Bacillus velezensis* FZB42	Alpha	BH	11.93
11.	*Bacillus bingmayongensis* FJAT-13831	Alpha	BS	11.24
12.	*Bacillus paranthracis* MCCC 1A00395	Gamma	BH	9.29
13.	*Bacillus marcorestinctum* LQQ	Alpha	BH	7.81
14.	*Bacillus thuringiensis* ATCC 10792	Gamma	BS	7.47
15.	*Bacillus albus* MCCC 1A02146	Alpha	BH	6.30

*BH: healthty soil; BS: Foc-infected soil

Discussion

Fusarium oxysporum f.sp. cubense is the most devastating fungal pathogen of banana plants which causing fusarium wilt disease. The disease currently has known spread widely in almost all banana production countries (Cheng et al, 2019). In our previous study, we compared diversity and abundancy of soil bacteria in healthy and Foc-infected soils using metagenomic data and we found that the healthy soil tends had lower species abundancy but

had higher diversity in species in comparison Foc-infected soils (Effendi et al, 2019). In this study, we continue our previous works by isolating and characterizing culturable-soil bacteria from both soil conditions.

The results of BLAST-N as shown in Table 3 indicated that all isolates have high score of similarity with NCBI database. The smallest value is 99.56% (BS 35B) and the largest value is 100% (BH 14A, BH 24A and BS 14B). The high similarity value and low E-value (0.0) from BLAST result indicate that all query sequence of the isolates have high homology with matched sequences in the NCBI database. With similarity value as mention above, we are confidence that all identified bacterial ID are correct. According Bordoni (2003), the results of searching for nucleotides with 70% identity percentage values have included significant results.

Most of bacterial isolates were identified in the genus *Bacillus* (88,5%) which consisted of 17 different species or strain. The rest were *Staphylococcus, Lysinibacillus*, and *Enterobacter*. Bacillus bacteria are easily found in soils because the soil is a reservoir of these bacteria (Watanabe and Hayano 1993). Indeed, *Bacillus* group has been identified as potential biological control agents against various pathogenic microbes (McSpadden Gardener and Fravel 2002; Romeis et al. 2006; Choudhary and Johri 2009). Soils with low organic matter are generally dominated by *Bacillus* species such as *B. subtilis, B. licheniformis,* and *B. cereus*, but more *Bacillus* species can be found also in soils with higher organic matter and fertility (Hardwood 1989).

Despite the functional role of *Bacillus* in the soil as Plant Growth Promoting Bacteria (PGPB) (Tilak and Ready 2006; Singh et al. 2008), *Bacillus* has role to control a wide range of plant pathogens. This broad spectrum of function comes from the ability of *Bacillus* to produces several novel metabolites or important functions that used for plant growth such production of IAA, fixation of N_2, phosphate solubilization, production of siderophore, HCN, and several important enzymes (Ongena et al. 2007; Singh et al. 2008; Chung et al. 2008; Chen et al. 2009; Arrebola et al. 2010). Thus, we performed functional character of isolated bacteria. All isolates that have been identified by using BLAST-N are then characterized their functional capabilities (Table 2). Isolate BS 15B is *Bacillus megaterium* strain NBRC 15308. In general, *B. megaterium* is a gram-positive bacteria, produces spores, and has habitats in various places such as soil, sea water, sediment, fish, and even food. *B. megaterium* has been used for around 50 years as an industrial microorganism, because it produces a variety of

products such as penicillin acylase, amylase, glucose dehydrogenase, vitamin B12 to fungicide toxins (Vary et al. 2007). Antibiotics are also produced by *B. megaterium* such as emimycin and oxetanocin which can function as antibacterial agents. Its function as a fungicide can reduce the infectivity of *Rhizoctonia solan*i in soybean plants, as well as increasing the growth of soybean plants (Vary 1994). Bacterial isolates BS 24D, BS 24F, and BS 26A are *B. thuringiensis* with different strains. *B. thuringiensis* produces endotoxins which are widely used as insecticides since the 1960s. The endotoxins produced are specific to a small subset of insects, and can be damaged if exposed to UV light and certain environmental factors (Waites et al. 2001). BS 26A isolate with the name *B. thuringiensis* strain NBRC 101235. BS 24D isolate is *Bacillus thuringiensis* strain IAM 12077. BS 24F is *Bacillus thuringiensis* strain ATCC 10792 was reported enable to control disease which caused by *Ralstonia* and *Fusarium* on chili plants (Yanti et al. 2018a). Whereas BS 25B is *Bacillus vallismortis* strain NBRC 101236 which is known to produce antimicrobial, one of which is Bacillomycin (Liu et al. 2017). The bacterial isolate of BS 25E is *Bacillus bingmayongensis* strain FJAT-13831. Based on research conducted by Yanti et al. (2018a), this bacterium can slow down the development time of Fusarium which attacks chili plants, as well as reduce the level of damage to infected plants.

Isolate BS 26B is *B. toyonensis* strain BCT-7112 which has been reported able to control *Ralstonia syzigii* subsp. Indonesiensis in potatoes and chili pepper (Yanti et al. 2018a). A consortia of *B. toyonensis* strain BCT-7112 with *B. pseudomycoides* strain NBRC 101232 (BS 35B), *B. cereus* strain CCM 2010 (BS 24C) had proved able to control wilting disease caused by *Ralstonia syzigii* subsp. Indonesiensis and increase the growth of tomato plants.

Next is the *Bacillus pseudomycoides* strain NBRC 101232 is anaerobic facultative gram-positive bacteria, with opaque white colonies and rhizoidal (like roots) which is common found in the soil (Nakamura 1998). Indeed, *B. pseudomycoides* strain NBRC has the ability to support the growth of tomato plants and control infection by *R. solanacearum* by reducing the severity or spread of wilted panyakit on plants (Yanti et al. 2018a).

BH 14C, BH 24A, BH 14A, BS 14B, and BS 24C are identidfied as *Bacillus cereus* strain IAM 12605, *Bacillus cereus* strain ATCC 14579, *Bacillus cereus* strain NBRC 15305, *Bacillus cereus strain* JCM 2152, *Bacillus cereus* strain CCM 2010, respectively. These bacteria were reported as endophytic bacteria from the Safflower plant and able to produces

IAA (Hemida & Reyad, 2019). B. cereus ATCC 14579 (BH 24A) had been demonstrated able controlling *R. solanacearum* infections and growth inducing of tomato plants (Yanti et al. 2018a). When it was inoculated together with *B. cereus* isolate NBRC 15305, they were able to induce growth increasing of chilli plants and inhibit disease infection by *Fusarium* and also *Ralstonia* (Yanti et al. 2018a).

B. cereus strain CCM 2010 (BS 24C) showed the same ability with *B. cereus* ATCC 14579 isolate (BH 24A) and *B. cereus* strain JCM 2152 (BS 14B) in increasing the growth of tomato plants and controlling of *R. solanacearum* (Yanti et al. 2018c). Moreover, application of bacterial consortia consisted of *B. cereus* CCM 2010 and *B. cereus* strain IAM 12605 (BH 14C) have reported able to control *Ralstonia syzigii* subsp. Indonesiensis and supports the growth of chili (Yanti et al. 2018b).

Bacillus velezensis strain FZB42 (BH 15C) showed ability producing of volatile organic compounds that are useful as biocontrol of plants against biotic stress (plant pathogens) such as against *R. solanacearum* (Fan et al. 2018). Some of these compounds include benzaldehyde, 1,2-benzisothiazol-3 (2 H) -one, and 1,3-butadiene. In addition, this bacterium can also produce Bacillomycin D which causes the death of hyphal cells and conidia of *F. graminearum,* thus it may inhibits panama wilt attacks on plants such as corn and wheat.

BH 16B is *Bacillus atrophaeus* strain JCM 9070 which was first found as endophytic bacteria in *P. cuspidatum* from Longquan and Qingchen. Sun et al. (2013) reported the *Bacillus atrophaeus* strain JCM 9070 was able to produce antifunctions such as hydrolytic enzymes, spore-specific lipopeptides, and fengysin. BH 34 A is *Bacillus marcorestinctum* strain LQQ that function as a biocontrol against spoilage in plants. Han et al. (2010) showed that the bacteria was able to control *P. carotovorum,* a decomposing pathogens, in potato tubers.

Some identifed bacteria had not been reported as unknown function bacteria. *Bacillus paranthracis* strain MCCC 1A00395 (BH 34C), *Bacillus mobilis* strain MCCC 1A05942 (BH 34D), *Bacillus proteolyticus* strain MCCC 1A00365 (BS 24B), *Bacillus pacificus* strain MCCC 1A06182 (BS 24E), *Bacillus proteolyticus* strain MCCC 1A00365 (BS 24B), *Bacillus pacificus* strain MCCC 1A06182 (BS 24E), *Bacillus proteolyticus* strain MCCC 1A00365 (BS 24B) (BH 16C) were first isolated from marine sediments (Liu et al. 2017). Then the isolate of the bacterium Bacillus tequilensis strain 10b (BS 14A), which was first discovered

in grave soil in the state of Jalisco, Mexico (Gatson et al. 2006). Whereas BS 35A *Staphylococcus arlettae* strain ATCC 43957 is a bacterium that is declared harmless, which can be found from the skin or nasal of birds (Castellanos-Arévalo et al. 2015).

But some identified bacteria have potential as pathogens not only for human but also for plants. *Bacillus cytotoxicus* strain NVH 391-98 is a pathogen that attacks humans. This bacterium is generally associated with food poisoning, which causes diarrhea and vomiting (Lapidus et al. 2008). Next is BS 34C isolate, *Lysinibacillus xylanilyticus* strain XDB9 which has the ability to degrade xylan. Xylan is a polysaccharide that is common in nature and is a component of hemicellulose which can be found in plant cell walls. So that these bacteria have the potential in the food industry and pulp or pulp (Lee et al. 2010). BH 15A bacterial isolate which is a *Enterobacter tabaci* strain YIM Hb-3 was found on the tobacco plant (Duan et al. 2015).

Some isolates were identified as *B. cereus* group, namely *B. cereus, B. thuringiensis* and *B. pseudomycoides* showed the potential to inhibit pathogenic fungal infections. The *B. cereus* group had known as producer a variety of toxins, bacteriocins and antibiotics that cause competition with other microorganisms. In addition to antibiotics, the *B. cereus* group also produces extracellular enzymes that function to degrade complex organic matter present in the soil, so that it benefits plants and soil fertility (Ceuppens et al. 2013).

Conclusion

Identification of culturable bacteria from health and Foc-infected soils in this study, according literatures, indicated that some identified soil bacteria have potency in inhibiting of bacteria and fungal pathogens such as *Ralstonia, Rhizoctonia*, and *Fusarium*. Thus, these bacteria may be used as biocontrol for Foc infections. Antagonistic test with Foc showed 13 strain of *Bacillus* and 2 other bacteria (*Lysinibacillus xylanilyticus* and *Staphylococcus arlettae)* have potential to control developing of Foc. The best inhibition was showed by *B. cereus* CCM 2010.

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