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Short Communication: Potential tests of plant growth bacteria for the control of *Peronosclerospora philipinensis* in corn

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Abstract. *Djaenuddin N, Syafruddin, Patandjengi B, Kuswinanti T. 2020. Short Communication: Potential tests of plant growth bacteria for the control of* Peronosclerospora philipinensis *in corn. Biodiversitas 21: 3886-3892.* The study was conducted at the Laboratory and Screen House of the Indonesian Cereals Research Institute (ICERI). The stages of the study were (i) potential test of bacterial isolates that have the ability to control downy mildew disease in vivo in corn and (ii) molecular identification of the selected bacterial isolates. Experiments were arranged in a Completely Randomized Design (CRD) with treatment of 24 bacteria which suspected to be growth-promoting bacteria. The parameters observed were disease intensity, percent disease suppression, plant height, chlorophyll content, and crop wet weight. The result showed that only five bacterial isolates namely, *Bacillus albus* strain MCCC 1A02146, *Bacillus cereus* strain IAM 12605, *Bacillus paramycoides* strain MCCC 1A04098, *Pseudomonas stutzeri* strain CCUG 11256, *Serratia marcescens* subsp. *sakuensis* strain KRED, have the ability to induce resistance to downy mildew disease caused by *Peronosclerospora philipinensis*.

Keywords: Corn, disease incidence, Downey mildew, induce resistance, *Peronosclerospora philipinensis*

INTRODUCTION

Downy mildew (DM) is one of the most harmful diseases of corn caused by *Peronosclerospora philipinensis* (Lantican et al. 2013). Of the 12 species of DM in corn, only three have been recorded as the most destructive species, namely *P. sorghi*, *P. phillipinensis*, and *P. maydis*. More research so far has focused on *P. sorghi* and *P. philipinensis* (Lukman et al. 2016). DM can cause crop damage up to 100%, especially on susceptible varieties, and markedly reduce the corn productivity in Indonesia (Pudjiwati et al. 2013). Synthetic pesticides are mostly used to control the disease. In addition to rising economic costs, improper control by synthetic pesticides also negatively impacts environmental sustainability (Meena et al. 2015).

Synthetic pesticides are able to suppress plant diseases, but damage beneficial microorganisms that are found in the soil and also pollute the soil environment (Tariq et al. 2017). To overcome the negative effects of the use of synthetic pesticides, it is necessary to have eco-friendly controls that produce healthy and high-quality plants. A possible alternative to control DM is eco-friendly plant growth-promoting rhizobacteria or PGPR.

In some studies, PGPR such as *Bacillus cereus, B. amyloliquefaciens, B. substilis, B. pasteurii, B. pumilus, B. mycoides,* and *B. sphaericus* have been used to control disease incidence and severity from various diseases in various plants (Resti et al. 2013). The use of *Bacillus* sp. can suppress DM on grapes caused by *Plasmopora viticola* (Zhang et al. 2017). However, detailed information about the ability of bacteria in controlling DM in corn is still limited. According to Mota et al. (2017), it is very important to develop a fast and efficient method for selecting bio-control microorganisms, especially when evaluating a large number of bacterial isolates. The discovery of bacterial isolates suitable for the treatment of corn seeds in controlling *P. philipinensis* is very significant in the field of biological control and development of biopesticide production in Indonesia. The aim of this study was to obtain bacterial isolates capable of controlling DM disease, stimulate the corn growth and molecular identification of these isolates.

MATERIALS AND METHODS

Study area

The research was carried out in the laboratory and screen house of ICERI at Maros from September to December 2019. A total of 24 bacterial isolates were isolated from various agricultural and plantations habitats of the South Sulawesi (Table 1).

Bacterial potential test in controlling downy mildew *in vivo*

Seed treatment

Maize seeds were soaked in selected bacterial suspensions) with a concentration of 10^9 cfu g^{-1} for two hours and then dried. For comparison, K1 with one type of synthetic pesticides (ai; metallaxyl) and K2 ie seeds soaked with sterile distilled water.

Table 1. Diversity of bacterial isolates collected from various agricultural and plantation habitats

Name of isolates	Name of collection sites
Ms-3	Maros
Ms-4	Maros
Ms-8	Maros
Be-2.1	Bone
Be-3	Bone
Be-4	Bone
Wo-2.1	Wajo
Wo-2.2	Wajo
Wo-3.1	Wajo
Wo-3.2	Wajo
Ga-1.2	Gowa
Ga-1.3	Gowa
Ga-2	Gowa
Ga-2.1	Gowa
Ga-2.2	Gowa
Ga-3	Gowa
Ga-3.1	Gowa
Si-1	Sinjai
Si-3	Sinjai
Si-4	Sinjai
Bg-1	Bantaeng
Bg-1.2	Bantaeng
Tp-2	Tanjung Pinang
Tp-3.1	Tanjung Pinang

Planting

Seeds were planted in micro plots (with an area of 5 m^2) in the screen house with a spacing of 75 cm × 25 cm with one seed per hole, so that there are 20 plants per row in one replication. The seeds used were Anoman variety. After being planted, at the age of 7-10 days after planting (DAP), spore inoculation was carried out by spraying a conidia suspension of *P. philippinensis* (10^6 spores/ml) on the leaves of corn plants at around 03.00-04.00 am. Inoculation was repeated three days later to get optimal symptoms. Urea fertilizer was applied as much as 150 kg/ha at 10 days after planting (DAP).

Molecular identification of bacterial isolates

Molecular identification was performed on selected bacterial isolates which have a potential to control DM disease in corn. Bacterial DNA was isolated and purified using the *Quick-DNA ™ Fungal/Bacterial Miniprep Kit* (D6005). The isolated DNA was then amplified using a pair of primers 27F (5'AGAGTTTGATCCTGGCTCAG 3') and primers 1 4 9 2R (5' TACGGYTACCTTGTTACGA CTT 3'). The conditions of PCR were as follows: set at a temperature of initial denaturation at 95˚C for 1 min, followed by 35 cycles of denaturation at 95˚C for 15 seconds, annealing at 55˚C for 15 seconds and finally extension at 72˚C for 42 seconds. The PCR result was then electrophoresed using *MyTaq HS Red Mix* (Bioline) and visualized under UV Transilluminator. Amplification of DNA from selected isolates produced a band that was ± 1400 bp. The sequencing of DNA was performed at Genetics Science Inc. Co. in Jakarta, Indonesia. Data sequencing results were matched with the *Gene Bank NCBI* using the *BLAST* on http://www.ncbi.nlm.nih.org.

Observation and data collection

Observations were done every day for three weeks to determine the incubation period of downy mildew. Other variables observed were (%) disease incidence at 21, 28, 35 DAP, percentage of disease suppression, and plant height (21 and 35 DAP). For chlorophyll, content leaves were sampled at the base, middle, and tip portions on 35 DAP, and the wet weight of corn plants was recorded at 49 DAP. Disease incidence was calculated according to the formula (Sekarsari et al. 2013):

$$DI\ (\%) = \frac{A}{B} \times 100$$

Where;

DI : DM incidence (%)

A : Number of DM-infected plants

B : Number of plants observed

Disease suppression percentage in disease incidence (DI) was determined according to the following equation:

Disease suppression % = {(DI in control %-DI in treatment %)/DI in control} x 100

Research design and data analysis

The experiments were arranged in a completely randomized design with the treatment of 24 bacterial isolates and 2 controls. The experiment was carried out in two replicates. The data were analyzed using one-way analysis of variance followed by LSD test at 5% significance level.

RESULTS AND DISCUSSION

Bacterial potential test in controlling downy mildew in vitro

The result showed that application of bacteria on seeds has a positive effect on the rate of germination. In some bacterial treatments namely Ga-1, Ga-2.2, Ga-3, Ms-8, Wo-2.1, Wo-3.1, and Wo-3.2 germination reaches up to 100%, whereas in the controls (K1 and K2) germination was <100%. Symptoms of downy mildew began to appear at 17 days after planting in the controls and bacterial treatments (Table 2).

Result of *in vivo* test of 24 bacterial isolates can be seen in Table 3. The incidence of DM in aqua control (K2) at 28 and 35 DAP was 52.8% and 89.7% respectively, while it was 44.1% and 59.1% in synthetic control (K1). The observation of the percentage of downy mildew infection starting 21 and 28 DAP has not been significant between bacterial treatments and control. However, at 35 DAP, twelve bacterial isolates had significantly lower disease intensity <7% with infection conditions in control of 89%.

All isolates were able to suppress downy mildew infection, indicating the average effectiveness of disease suppression as compared to control. The effectiveness of disease suppression ranges from 2-100%. The treatment of six bacterial isolates i.e. Bg-1, Ga-2, Ga-3, Si-4, Tp-2, and

Tp-3.1 was found to be effective in controlling downy mildew disease and even symptoms did not appear at 35 DAP, which means that these isolates have the ability to suppress downy mildew disease up to 100%. Bg-1.2, Ga-2.1, Ga-2.2, Ga-3.1, and Si-3 were the other bacterial isolates, with infection rate ranging from 2.9 to 6.3% and suppression rate between 88 and 97% (Figure 1).

The effect of bacterial isolates on the growth of corn plants can be seen in Table 4. Results from 24 bacterial isolates showed that they did not have a negative effect on plant growth and average plant height was not significantly different from controls. While in many isolates, chlorophyll content was significantly different from the controls. This was related to the rate of downy mildew infection in each treatment. Among the 24 bacterial isolates, the highest chlorophyll content was recorded in Si-4 (43), followed by Ga-3.1 (41.9), Si-3 (41.2), Ga-2.1 and Ga-2 each (41.0), Tp-2 and Bg-1 each (40.9) and Si-1 (40.8), whereas in aqua control and synthetic control (K1and K2) it was 24.7 and 29 respectively. The highest 5.48 kg wet weight was recorded in Si-1, followed by 5.37 kg in Ga-3 and 5.15 kg in Ga-2.1 bacterial isolate. While wet weight was 3.20 kg and 3.27 kg in aqua control (K2) and synthetic control (K1) respectively. The six isolates that were able to suppress the infection significantly had higher wet weight than controls. It was also recorded that not all the bacterial isolates were capable to trigger plant growth, as their results were lower than controls.

Identification of bacterial isolates

Six isolates namely Bg-1, Ga-2, Ga-3, Si-4, Tp-2, and Tp-3.1 were selected for molecular identification as they found to be effective in DM suppression. Amplification of DNA from 6 selected isolates produced a band that was ± 1400 bp. After the sequencing of nucleic acid and BLAST analysis, the isolated identities were obtained as in Table 5. Phylogenetic analysis and the alignment of 16s rDNA gene sequences based on the 16s rDNA gene library was shown in Figure 2.

Results of BLAST analysis of Bg-1 isolate had 99.7% similarity with *Bacillus albus* strain MCCC 1A02146, Ga-2 and Si-4 isolates had 99% similarity with *Bacillus cereus* strain IAM 12605, Ga-3 isolates had 99.8% similarity with *Bacillus paramycoides* strain MCCC 1A04098, Tp-2 isolates had 99.8% similarity with *Pseudomonas stutzeri* strain CCUG 11256 and Tp-3.1 isolate had 99.6% similarity with *Serratia marcescens* subsp. *sakuensis* strain KRED (Table 5).

Seed treatment is thought to systemically protect plants from pathogenic infections so as to reduce the incidence of disease at the beginning of planting. The lowest disease incidence was recorded in Bg-1, Ga-2, Ga-3, Si-4, Tp-2, and Tp-3.1 isolates which were significantly different from controls. These were the six best isolates to suppress the disease severity of downy mildew on maize, which was significantly different from controls and other treatments.

Table 2. Percentage of seed germination and incubation period of downy mildew in different treatments

Treatment	Germination (%)	Incubation period (days)
Be-2.1	94	18abc
Be-3	93	18.5 abc
Be-4	98	18.5 abc
Bg-1	90	0-c
Bg-1.2	94	31.5 a
Ga-1.2	91	18.5 abc
Ga-1.3	98	19.5 abc
Ga-2	81	0-c
Ga-2.1	100	14 abc
Ga-2.2	100	14 abc
Ga-3	100	0-c
Ga-3.1	90	21 abc
Ms-3	88	17 abc
Ms-4	92	17 abc
Ms-8	100	18 abc
Si-1	95	28 ab
Si-3	97	21 abc
Si-4	92	0-c
Tp-2	95	0-c
Tp-3.1	94	0-c
Wo-2.1	100	17 abc
Wo-2.2	94	19 abc
Wo-3.1	100	18 abc
Wo-3.2	100	20.5 abc
K1/synthetic fungicide	97	6 bc
K2/aquadest	95	5 bc

The numbers in the same column followed by the same letter are not significantly different according to the LSD test at α = 0.05

Table 3. Development of downy mildew disease in various DAP in different treatments

Treatments	DM incidence (%) at		
	21 DAP	28 DAP	35 DAP
Be-2.1	2.2 ab	46.7 bcde	69.6defg
Be-3	14.3 abc	54.8 bc	81.3 efg
Be-4	3.7 ab	28.1 abcde	52.6 def
Bg-1	0.0 a	0.0 a	0.0 a
Bg-1.2	2.9 ab	6.3ab	6.3ab
Ga-1.2	1.5 a	26.4 abcde	60.8 defg
Ga-1.3	6.9 abc	13.9 abcd	38.5 bcd
Ga-2	0.0 a	0.0 a	0.0 a
Ga-2.1	2.6 ab	5.3 ab	5.3 ab
Ga-2.2	2.8 ab	2.8 ab	5.6 ab
Ga-3	0.0 a	0.0 a	0.0 a
Ga-3.1	0.0 a	0.0 a	2.9 ab
Ms-3	20.8 bc	60.7 e	87.5fg
Ms-4	5.1 abc	30.9 abcde	66.3 defg
Ms-8	1.7 a	51.7 cde	72.4 defg
Si-1	5.6 abc	10.8 abc	10.8abc
Si-3	0.0 a	2.9 ab	2.9ab
Si-4	0.0 a	0.0 a	0.0 a
Tp-2	0.0 a	0.0 a	0.0 a
Tp-3.1	0.0 a	0.0 a	0.0 a
Wo-2.1	23.7 c	44.6 bcde	50.6def
Wo-2.2	0.0 a	30.0 abcde	45.9cde
Wo-3.1	6.3 abc	32.2 abcde	67.8 defg
Wo-3.2	2.0a	23.9 abcde	47.4 cde
K1/synthetic	1.6 a	44.1 bcde	59.1 defg
K2/aquadest	17.0 abc	52.8 cde	89.7g

The numbers in the same column followed by the same letter are not significantly different according to the LSD test at α = 0.05

Table 4. Effect of 24 bacterial isolates on plant height, chlorophyll content and wet weight of corn plants

Treatment	Plant height (cm)		Chlorophyll content (units) at 35 DAP	Wet weight (kg)
	21 DAP	35 DAP		
Be-2.1	72.0 a	123.4 a	28.3 bcd	3. 51 abc
Be-3	71.9 a	114.3 a	24.1 d	2. 86 abc
Be-4	74.9 a	123.1 a	28.7 bcd	3.64 abc
Bg-1	71.8 a	124.7 a	40.9 a	4. 18 abc
Bg-1.2	69.1 a	129.3 a	39.5 a	4. 37 abc
Ga-1.2	74.5 a	124.4 a	23.7 d	3. 73 abc
Ga-1.3	71.8 a	123.9 a	30.7 b	3. 04 abc
Ga-2	77.0 a	124.3 a	41.0 a	4, 30 abc
Ga-2.1	71.1 a	127.0 a	41.0 a	5. 15 ab
Ga-2.2	71.4 a	129.1 a	38.4 a	4. 37 abc
Ga-3	72.2 a	139.8 a	39.4 a	5. 37 a
Ga-3.1	73.8 a	126.2 a	41.9 a	4. 40 abc
Ms-3	71.9 a	119.4 a	24.7 cd	3. 20 abc
Ms-4	76.0 a	134.3 a	28.8 bcd	3. 99 abc
Ms-8	73.2 a	123.1 a	26.6 bcd	3. 37 abc
Si-1	72.9 a	129.1 a	40.8 a	5. 48 a
Si-3	72.9 a	116.5 a	41.2 a	2. 87 abc
Si-4	66.6 a	131.2 a	43.2 a	4. 32 abc
Tp-2	73.6 a	122.6 a	40.9 a	3. 74 abc
Tp-3.1	74.1 a	136.5 a	38.6 a	4. 87 ab
Wo-2.1	6 7.8 a	112.2 a	27.3 bcd	2. 09 c
Wo-2.2	75.2 a	128.2 a	27.7 bcd	2. 66 bc
Wo-3.1	73.5 a	118.0 a	30.1 bc	2. 98 abc
Wo-3.2	76.7 a	127.8 a	28.1 bcd	3. 45 abc
K1/synthetic	70.9 a	131.3 a	29.0 bcd	4. 00 abc
K2/aquadest	64.0 a	115.5 a	24.7 cd	3. 27 abc
CV (%)	7.6	10.1	7.1	28.6

Note: The numbers in the same column followed by the same letter are not significantly different according to the LSD test at $\alpha = 0.05$

Table 5. Results of BLAST analysis of bacterial isolates

Isolate code	Identity	% similarity
Bg-1	*Bacillus albus* strain MCCC 1A02146	99.74
Ga-2	*Bacillus cereus* strain IAM 12605	99.93
Ga-3	*Bacillus paramycoides* strain MCCC 1A04098	99.81
Si-4	*Bacillus cereus* strain IAM 12605	99.65
Tp-2	*Pseudomonas stutzeri* strain CCUG 11256	99.86
Tp-3.1	*Serratia marcescens* subsp. *sakuensis* strain KRED	99.65

Discussion

The bacterial application had a slower incubation period than the controls, even some bacterial treatments were not affected by the fungus. DMs colonize freely in plants in the absence of other microorganisms that lead to a short incubation period for disease and appearance of early symptoms (Kaur et al. 2011). Plants that have a longer incubation period potentially involving the speed of the plant in activating its defence system which is influenced by the bacteria applied. Using bacterial strains in plants was very important because these microorganisms were able to establish relationships with plants and stimulate plant growth through many beneficial physiological characteristics (Souza et al. 2015). Treatment of antagonistic bacteria can provide a defense system (bioprotectant), as these bacteria secrete antibiotic compounds that were able to signal affected plants. Rhizosphere bacteria act as biological agents through the production of antibiotics, lytic enzymes, hydrogen cyanide, siderophore, or competition for nutrition and space (Raj et al. 2012).

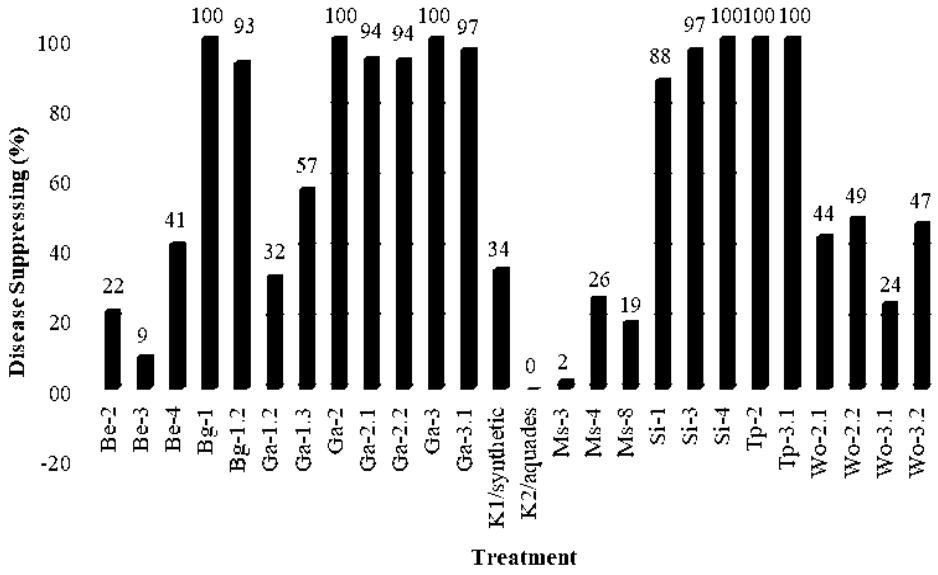

Figure 1. Percentage of downy mildew suppression in various treatments

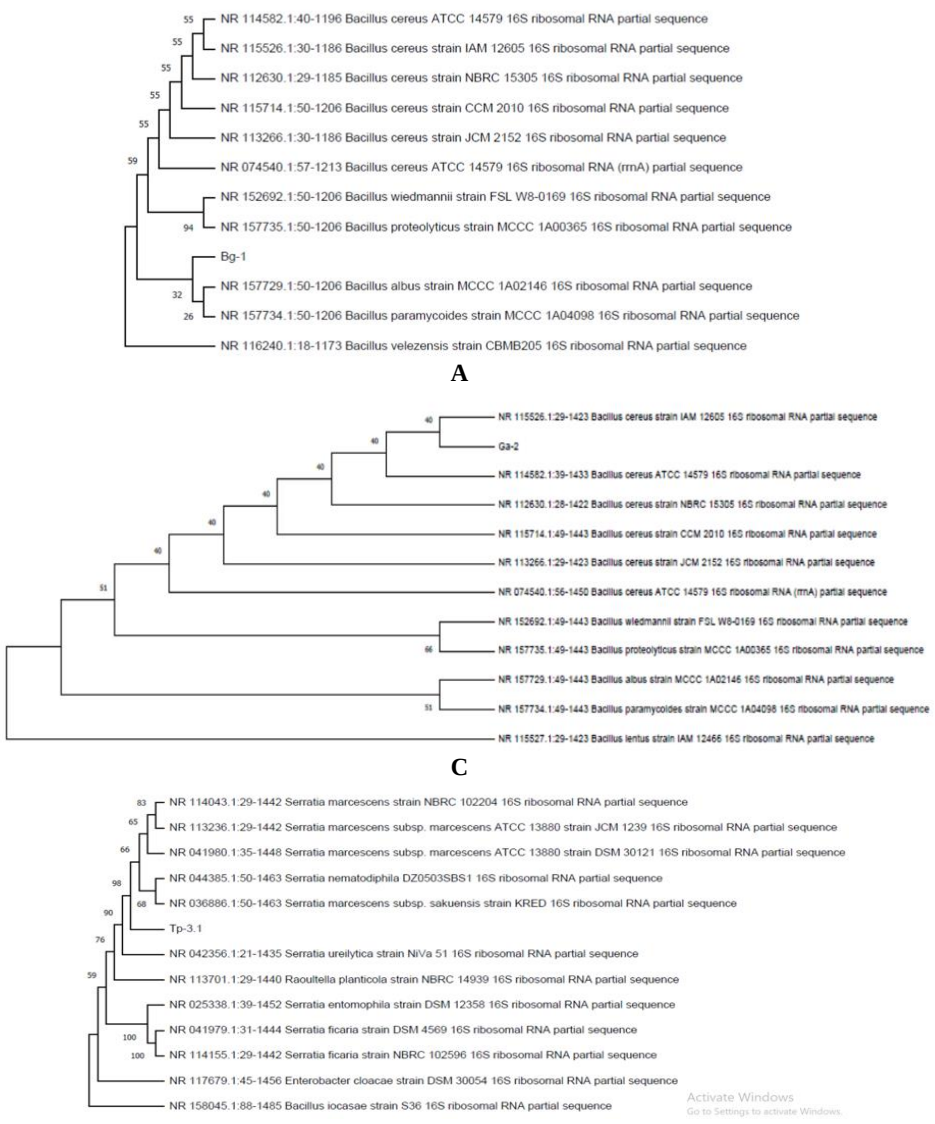

Figure 2. Phylogenetic trees of Bg-1 (A), Si-4 (B), Ga-2 (C), Ga-3 (D), Tp-3.1 (E), and Tp-2 (F), and group-with (group) trees his relatives

Treatment of bacterial isolates can suppress DM infection in corn plants. The effective suppression of DM by isolates of Bg-1, Ga-2, Ga-3, Si-4, Tp-2 and Tp-3.1 was up to 100% at 35 DAP. The DM pressure was related to the ability of a bacterium to colonize the leaves and produce secondary metabolic compounds that can protect plants from pathogens. This similar result was obtained by Suryadi et al. (2013) that metabolic products produced by bacteria can inhibit disease incidence in plants. Bacteria can enter through the process of seed germination, secondary roots, stomata or injured leaves (Resti et al. 2013).

Downy mildew infection was negatively correlated with leaf chlorophyll. Plants treated with Bg-1, Ga-2, Ga-2.1, Ga-3.1, Si-1, Si-3, Si-4, Tp-2, and Tp-3.1 bacterial isolates have lower DM incidence and high chlorophyll content than controls. DM infection decreases the chlorophyll content in corn plants. Hao et al. (2013) reported that DM infection reduced the photosynthetic rate and chlorophyll content in plants. The treatment of bacterial isolates showed that the results of the analysis were not significantly different from the control on aspects of plant height, but the fresh weight of the plants showed different results in each treatment of bacterial isolates, this was thought to be related to the different mechanism of action of each isolate shown by its ability to dissolve phosphate, potassium, and nitrogen-fixing. The results of the study by Manzoor et al. (2016) showed that the phosphate solvent bacteria were able to increase the biomass of corn plant and accumulation of phosphorus in plants. *Pseudomonas fluorescens* was capable of producing plant growth-enhancing agents and antifungal substances, for the development of biological fertilizers and bioinoculants for food crops (Noori and Saud 2012).

Several bacterial genera have been used as biocontrol agents to control DM disease. The genus *Bacillus* and *Serratia* have been reported to control DM in cucumbers for controlling DM in cucumbers (Sun et al. 2013; Tesfagiorgis et al. 2014; Mohamed et al. 2016). Raj et al. (2017) reported that *Bacillus strains* can induce resistance in millet to control DM. Genus *Pseudomonas* has the potential to control downy mildew on mollusks and mustard greens (Elsharkawy et al. 2014; Damiri et al. 2017).

In conclusion, the Bg-1, Ga-2, Ga-3, Si-4, Tp-2, and Tp-3.1 isolates were able to suppress DM infection and increase corn yield by up to 100%. The selected bacterial isolates were *Bacillus albus* strain MCCC 1A02146, *Bacillus cereus* strain IAM 12605, *Bacillus paramycoides* strain MCCC 1A04098, *Pseudomonas stutzeri* strain CCUG 11256, *Serratia marcescens* subsp. *sakuensis* strain KRED. These isolates can be used as PGPR and biological pesticides to control downy mildew disease on corn.

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