Ecological and physiological characterization of ACC deaminase-producing bacteria isolated from specific sites in Yogyakarta, Indonesia

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Abstract. The decrease of plant growth and productivity may be due to environmental stresses that induce excessive ethylene synthesis. ACC (1-aminocyclopropane-1-carboxylate) deaminase-producing bacteria are known to decrease plant ethylene levels by converting the ethylene precursor, ACC to ammonia and α-ketobutyrate. This study was aimed to understanding ecological and physiological characters of ACC deaminase-producing bacteria. The bacterial strains used were *Sphingobacterium multivorum* BK1, *Bacillus mycoides* CB2, *Pantoea dispersa* CK4, *Pantoea agglomerans* KD6.2, *Enterobacter ludwigii* KW3, *Bacillus aryabhattai* TW7, *Pseudomonas monteilii* KS12, *Pseudomonas putida* KS16.2, *Pseudomonas putida* PIR 3C, *Stenotrophomonas maltophilia* PIR5, *Lysinibacillus pakistanensis* PIC5, *Bacillus aryabhattai* PIC11, and *Roultella terrigena* PCM8. It was observed that all bacterial strains demonstrated optimal growth at pH 6-8, temperature 25–30˚C, and oxygen requirement of aerobic to facultative anaerobic. All of strains have Phosphate Solubilizing Index between 1,1–3,04 and IAA (Indole-3-Acetic Acid) production level between 0,11-10,33 ppm.hour-1. In terms of antagonistic properties, all bacterial strains were found incapable of inhibiting *Xanthomonas oryzae pv. oryzae* and *Ralstonia solanacearum* growth. All bacterial strains were capable of using glucose, sucrose, cellulose and mannitol as the source of carbon and energy. In addition, all bacterial strains also demonstrated Cr6+ tolerance ofs 100–300 mg.L-1.

1. Introduction

Agriculture is an important aspect to providing sufficient food for human in the world. The ever-increasing world population lead to increasing food demand, but the food production availability is insufficient. This is due to the decreasing productivity of plants.

The productivity of plants is increasingly affected by biotic and abiotic stresses. Abiotic stresses on environmental conditions include water (excess or lack), salinity, extreme temperatures, heavy metals and ultraviolet radiation, while biotic stresses are caused by damage due to attacks by nematodes and pathogenic microorganisms (bacteria, fungi, viruses). Biotic and abiotic stresses cause plants to induce excess ethylene synthesis, thus inhibiting growth and decreasing plant productivity. At low levels, ethylene plays a role in stimulating plant development [1].

Ethylene levels in plants can be suppressed by the activity of promoting bacterial plant growth (PGPB) by producing the ACC deaminase enzyme. The mechanism and role of PGPB to promote and helping increasing the plant growth have been studied since the last 15-20 years. PGPB are associated with plants in several ways, including around the roots of plants (rhizosphere), endophytes, symbiosis and also in part of the leaf surface (phyllosphere). PGPB has been widely applied to several types of plants, including food crops, vegetables, and fruits and have a positive impact on plant growth and yield [2].

The bacteria which symbiotic with plants will facilitate the development of host plants where bacteria live. Some of these bacteria can contain ACC deaminase to reduce stress levels of ethylene plants, facilitate dissolution of minerals such as phosphorus from the soil which then becomes more readily available for plant growth, and synthesize phytohormones, especially IAA which can function to increase various stages of plant growth.

Previous studies have isolated and selected ACC deaminase-producing bacteria. The bacteria are isolated from several plant commodities, including food crops (paddy), horticultural crops (chili, potatoes, shallots), and plantation crops (tea, cocoa). This research is directed to study the ecological and physiological character of these isolates in order to further study about the potential of isolates as biological fertilizer inoculums in stressed environment conditions.

2. Materials and Methods

2.1. Bacterial strains

All of bacterial strains are an ACC deaminase producing bacteria isolated from Special Region of Yogyakarta. The bacteria were *Sphingobacterium multivorum* BK1, *Bacillus mycoides* CB2, *Pantoea dispersa* CK4, *Pantoea agglomerans* KD6.2, *Enterobacter ludwigii* KW3, *Bacillus aryabhattai* TW 7, *Pseudomonas monteilii* KS 12, *Pseudomonas putida* KS 16.2, *Pseudomonas putida* PIR 3C, *Stenotrophomonas maltophilia* PIR 5, *Lysinibacillus pakistanensis* PIC 5, *Bacillus aryabhattai* PIC 11, and *Raoultella terrigena* PCM 8. The bacteria were subcultured on Nutrient Agar medium.

2.2. Antibiotic resistance assay

To test antibiotic resistance, the bacterial strains were tested against ampicillin, kanamycin, and ciprofloxacin. Bacterial cells were cultivated by streaked plate method on nutrient agar (NA) media supplemented with serial concentration of each antibiotic. The minimum inhibitory concentration (MIC) of bacterial strains against the three antibiotics determined using the microdilution method as described by Jorgensen et al. [3]. Each antibiotic were serial diluted into a series of concentration from 0 ppm to 200 ppm. The culture media were incubated at room temperature for 168 hours and each culture media was made in duplicate. The lowest concentration of antibiotics that inhibited the visible growth of the bacterial strain was recorded as the MIC value.

2.3. Effect of pH on the growth of ACC deaminase-producing bacteria

The pH effect assay was determined using Nutrient Agar (NA) medium which was set at pH values of 4, 5, 6, 7, 8 and control (pH 6.5). Treatment of various pH in the medium was prepared by adding 0.5 M HCl solution for low pH, and NaOH for alkaline pH and control without the addition of HCl or NaOH. pH measurements are carried out using a pH meter (Ohaus). Then the bacterial strains inoculated in the medium by a streak plate method, and incubated for 24-48 hours at room temperature and observed its growth [4].

2.4. Effect of temperature on the growth of ACC deaminase-producing bacteria

This assay was carried out by observing the growth of bacterial strains on medium with various temperature treatments. One bacterial culture was inoculated into 3 ml of Tryptic Soy Broth (TSB) medium as a pre-culture, then shaken for 24-30 hours. A total of 1% of bacterial cultures from pre-culture were inoculated on TSB medium, then incubated at 16, 20, 25, 30, 35, and 40ºC in the waterbath

during the mid-log phase (24-30 hours). Bacterial growth was observed spectrophotometrically at 600 nm [4].

2.5. Aerobicity assay of ACC deaminase-producing bacteria

The aerobicity assay of bacterial strains were carried out using semi-solid tioglycolate medium. A total of 5 ml semi-solid thioglycolate medium in a test tube was inoculated with a bacterial strains culture, then covered with 0.5 cm of mineral oil on the upper part of the medium and incubated for 24-48 hours at room temperature. The growth of bacterial colonies evenly shows that bacteria are facultative, growth on the base of the medium indicates that the bacteria are obligate anaerobic, and growth on the upper part of the medium indicates that the bacteria are aerobic [5].

2.6. Phosphate (P) solubilizing activity

Bacterial strains were inoculated in spot inoculation on Pikovskaya medium that containing $Ca_3(PO_4)_2$ (5g/l of distilled water), then incubated for 3-7 days at room temperature. The bacteria strains that are able to dissolve P are characterized by the development of clear zones around the growing colonies, then an analysis of phosphate solubilizing ability is carried out [6]. Analysis of phosphate solubilizing ability is calculating by the phosphate solubilizing index using the following formula [7]:

$$\text{Phosphate Solubilizing Index} = \frac{\text{Diameter of Clear Zone (cm)}}{\text{Colony Diameter (cm)}} \quad (1)$$

2.7. IAA production assay

Production of IAA by bacteria was evaluated as described by the Gordon and Weber method [8]. Tubes containing 3 ml of luria bertani broth with 100 µg/ml tryptophan were inoculated with bacterial cultures (107-108 cfu.ml-1) individually and incubated under shaking (120 rpm) conditions at 28 °C for 24 h. After incubation, the cultures were centrifuged at 5000 rpm for 15 min at room temperature. Then 2 ml of Salkowski reagent was added to 2 ml of culture supernatant and the resulting mixture was incubated at 30°C for 30 min in dark room. Development of pink colour indicates IAA is produced by the test bacteria. Absorbance was recorded at 530 nm using UV/VIS spectrophotometer [9]. Determination of IAA production in the sample was carried out by calculating the IAA value of sample in the IAA standard curve. The pellet is dried in an oven until the weight is constant to determine the dry weight of the cell.

2.8. Carbon sources utilization for the growth of ACC deaminase-producing bacteria

Carbon sources utilization ability is done by adding glucose, mannitol, cellulose and sucrose as much as 1% at a minimum medium (MM). One bacterial culture was inoculated into 3 ml TSB medium as a pre-culture, then shaken for 24-30 hours. Furthermore, the pre-culture was centrifuged at 13000 g for 4 minutes, resuspended 3 times using physiological salt (0.85% NaCl), and measured the density of bacterial colonies at 610 nm (OD 0.6 = 95µl). Bacterial culture was inoculated into 3 ml of MM with each carbon source as many as 3 replications and shaken during the mid-log phase (24-30 hours). After the incubation period, observation of the carbon sources utilization was carried out by measuring the density of bacterial colonies using a spectrophotometer at 590 nm [10].

2.9. Antagonistic activity of ACC deaminase-producing bacteria

Antibacterial activities were evaluated by inoculating *Xanthomonas oryzae pv. oryzae* (pathogenic bacteria) and *Ralstonia solanacearum* on the NA medium using a pour plate method, then the ACC deamination-producing bacterial strains were grown by point inoculation method. The culture incubated at room temperature for 24-48 hours and observed inhibitory zones formed. The inhibition zone formed can be calculated by the following formula [11]:

$$\text{Inhibitory Zone Index} = \frac{\text{Diameter of Inhibitory Zone (cm)}}{\text{Colony Diameter (cm)}} \quad (2)$$

2.10. The effects of heavy metals Cr $^{6+}$ on the growth of ACC deaminase-producing bacteria
The effect of heavy metals was done by inoculating bacterial culture on LB agar medium containing Cr^{6+} ($K_2Cr_2O_7$) with streak plate method at concentrations of 0, 5, 25, 50, 100, 150, 200, 250, and 300 ppm. Then incubated at room temperature for 24-48 hours and the growth of bacteria strains were observed [12]. The growth of bacterial strains on LB agar medium with various Cr^{6+} concentration is qualitative observation. The number of positive (+) mark indicated quality of the bacterial growth.

3. Results and Discussion

In order to further discriminate among the bacterial strains and as well as with other common endophytic bacteria, we have tested the selective resistance of each bacterial strains against certain antibiotics. Ampicillin, kanamycin, and ciprofloxacin were used for the antibiotic resistance test. The antibiotic resistance test, which was expressed as minimum inhibitory concentration (MIC) indicated that the ciprofloxacin and kanamycin were the best candidate as selective marker, because of lower MIC value compared to the ampicillin (Figure 1).

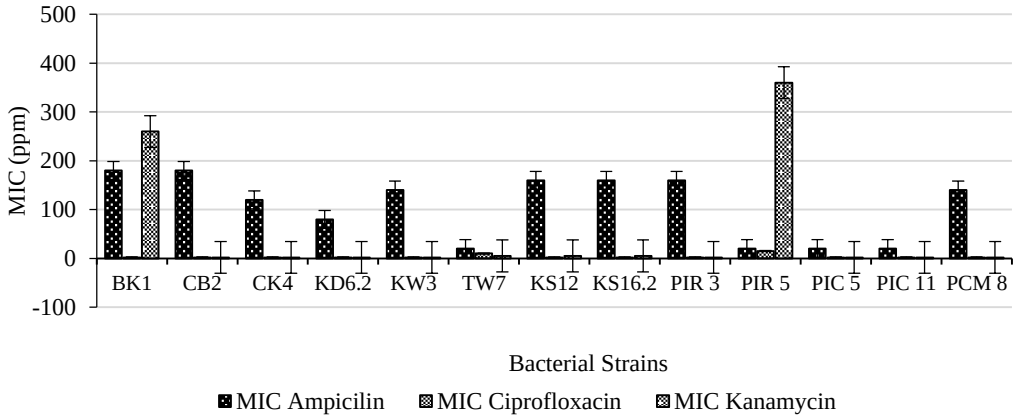

Figure 1. Antibiotic resistance of bacterial strains against ampicilin, ciprofloxacin, and kanamicin. Antibiotic resistance was calculated based on the minimum inhibitory concentration (MIC). Antiobiotic concentrations were varied from 0-200 ppm.

3.1. Ecology characterization of ACC Deaminase-Producing Bacteria
Bacterial growth is influenced by several ecological factors. Some ecological factors that influence bacterial growth include pH, temperature and oxygen. The following are the results of the growth of ACC deaminase producing bacteria that are treated with ecological factors.

3.1.1 Effect of pH on bacterial growth. Based on Figure 2, all of bacterial strains are able to grow well in the range of pH 6-8, while at pH 5 only a few strains are able to grow, and there is no growth at pH 4. Bacteria require physiological pH in cells for metabolic processes. Changes in the pH value of the medium lead to changes in the biology, physics, and chemistry reaction of bacterial and can inhibit the growth of some non-tolerant microorganisms [13].

Decreasing bacterial growth due to low pH conditions can be caused by cell energy to form a proton motif gradient from outer of cell into cell membrane. This causes partial inhibition of growth due to the cell secreting extra energy to maintain the potential of cell membrane. Furthermore, many enzymes for the activity of bacteria are sensitive to low pH [14].

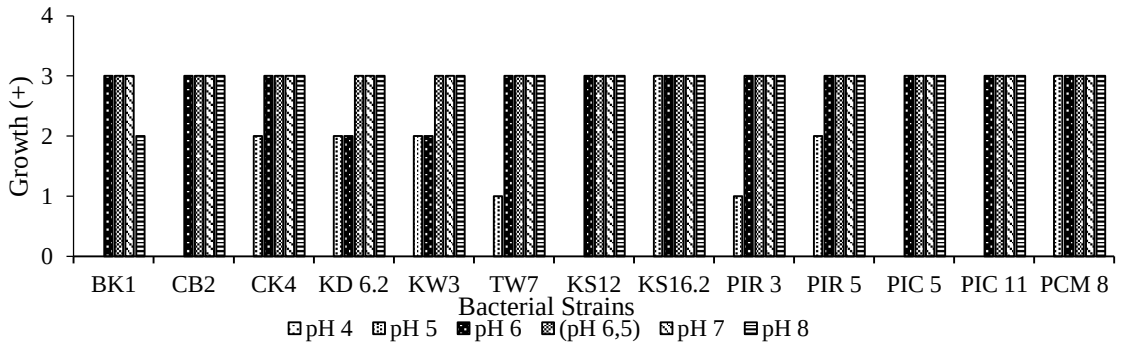

Figure 2. The qualitative growth of bacterial strains on nutrient agar medium added with HCl or NaOH with various pH medium. The number of positive (+) mark indicated quality of the bacterial growth.

3.1.2. Effect of temperature on bacterial growth. The growth of each bacterium under various temperature conditions is shown in Figure 3. Temperature is one of the ecological factors that influence the regulation of enzymes for the growth and activity of microorganisms. Based on Figure 3, all of bacterial strains grow on growth media at various incubation temperatures. The highest growth of bacterial strains occurred at temperatures between 25-30˚C, while at an incubation temperature > 30˚C, the bacterial growth was decreased based on absorbance measurements. Some bacterial strains can grow well at temperatures of 40˚C, namely KS12, KW3 and TW7.

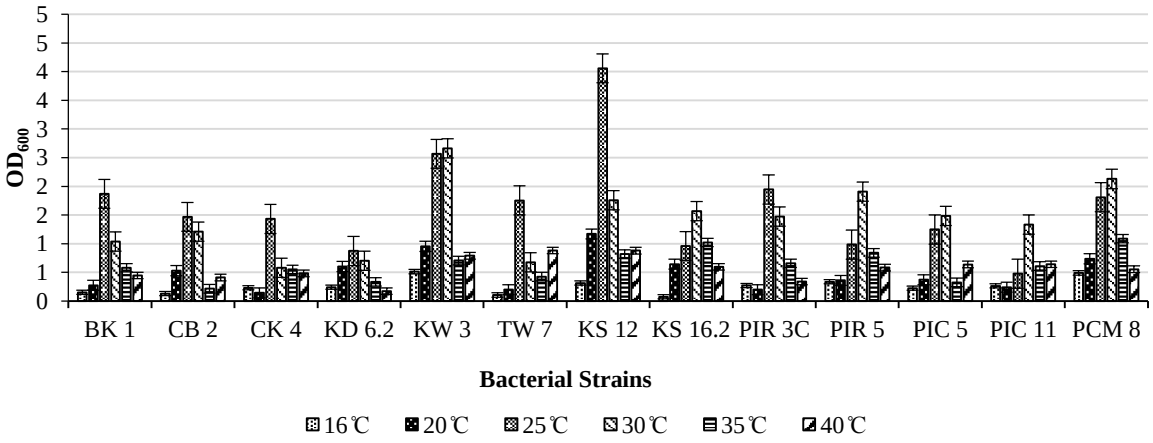

Figure 3. The qualitative growth of bacterial strains in TSB medium at various temperatures.

According to [15] and [16], most soil bacteria have optimum growth at temperatures more than 30˚C. An increase or decrease in temperature affects enzyme activity. The decrease in enzyme activity will affect the activity and growth of bacteria that cannot adapt to changes in temperature.

3.1.3. Aerobicity assay. The aerobicity assay of ACC deaminase-producing bacteria was carried out by puncture method on semi-solid tioglycolate medium and the surface of the medium was covered with mineral oil. The provision of mineral oil aims to minimize oxygen entering the medium. The aerobicity of ACC deaminase-producing bacteria is shown in Table 1.

Oxygen is one of the abiotic factors and components of bacterial cells whose numbers are affected by H_2O molecules. Some types of bacteria need oxygen for cell growth and activity. The oxygen requirement for cell growth and activity of each bacterium is different. Bacteria use oxygen as an electron acceptor for cell metabolic activity [17].

Table 1. Aerobicity trait of ACC deaminase-producing bacteria

No.	Bacterial Strain	Aerobicity trait
1.	*S. multivorum* BK1	Aerobe
2.	*B. mycoides* CB2	Facultative anaerobe
3.	*P. dispersa* CK4	Aerobe
4.	*P. agglomerans* KD6.2	Facultative anaerobe
5.	*E. ludwigii* KW3	Facultative anaerobe
6.	*B. aryabhattai* TW7	Aerobe
7.	*P. monteilii* KS12	Aerobe
8.	*P. putida* KS16.2	Aerobe
9.	*P. putida* PIR3C	Aerobe
10.	*S. maltophilia* PIR5	Aerobe
11.	*L. pakistanensis* PIC5	Aerobe
12.	*B. aryabhattai* PIC11	Aerobe
13.	*R. terrigena* PCM8	Facultative anaerobe

Oxygen is one of the abiotic factors and components of bacterial cells whose numbers are affected by H_2O molecules. Some types of bacteria need oxygen for cell growth and activity. The oxygen requirement for cell growth and activity of each bacterium is different. Bacteria use oxygen as an electron acceptor for cell metabolic activity [17].

Based on the results of the aerobicity test in Table 1, bacterial strains are aerobic and facultative anaerobic. Aerobic group bacteria are a group of bacteria that need oxygen for growth. Oxygen functions as an electron acceptor terminal during the process of aerobic respiration. Facultative anaerobic bacteria are a group of bacteria that can grow with the presence or absence of oxygen, because these bacteria can process cell metabolism in aerobic or anaerobic conditions [18].

3.2. Characterization of ACC deaminase-producing bacteria physiology

Bacteria are able to perform several physiological mechanisms to support cell growth. Some of the mechanisms carried out include phosphate dissolution, utilization of carbon sources, tolerance to heavy metals and competition with pathogenic microorganisms. The following is explained some of the characterizations of ACC deaminase-producing bacteria physiology.

3.2.1. Phosphate solubilization. Bacterial strains were characterized for their phosphate (P) solibilizing activity by the formation of visible dissolution halos on Pikovskaya medium contain tri calcium phosphate $Ca_3 (PO_4)_2$. The ability of phosphate solubilizing of ACC deaminase producing bacteria is shown in Figure.4.

The phosphate solubilizing of ACC deaminase producing bacteria were tested qualitatively and it was found that out of 13 bacterial strains, only 9 strains were able to solubilize phosphate, namely *B. aryabhattai* TW7, *P. putida* KS16.2, *P. dispersa* CK4, *P. agglomerans* KD6.2, *S. maltophilia* PIR5, *R. terrigena* PCM8, *P. monteilii* KS12, *E. ludwigii* KW3 and *P.putida* PIR3C with the highest solubilization index is P. agglomerans KD6.2.

Phosphate solubilization is one of the directly mechanisms by PGPB for promoting the plant growth [2]. Phosphate solubilizing microorganisms are able to solubilizing the insoluble phosphate by producing organic acids. Organic acids produced include acetic, lactic, malic, oxalic, succinic, citric, gluconate, ketogluconate and so on. The organic acids producing is carried out through 3 principles, namely competitive absorption reaction, formation of stable complexes with absorption of organic acids, and modification of exchange surfaces on orthophosphate uptake by organic ligand adsorption. Increased organic acids lead to decreasing in pH, resulting in the dissolution of P bound by Ca. The

genera of PGPB that capable to solubilize phosphate include Bacillus, Pseudomonas, and Enterobacter [19]; [20]. Application of PGPB that have phosphate solubilizing ability may be helpful to efficiency of plant to use and absorb large amount of phosphates.

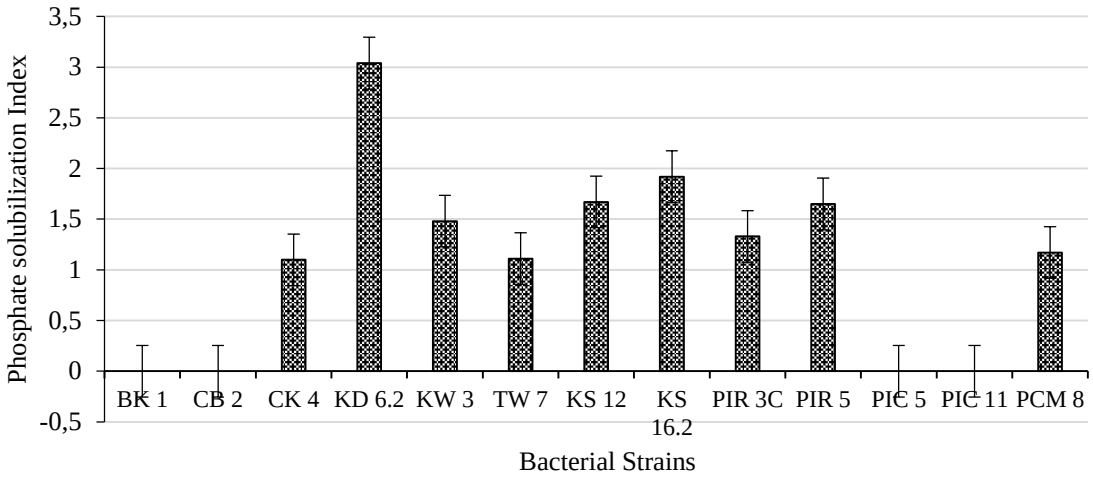

Figure 4. Phosphate solubilization index of ACC deaminase producing bacteria.

3.2.2. IAA production assay. PGPB directly promote plant growth through the production of plant hormone particularly IAA. IAA has a crucial role in plant root formation and cell division stimulation even though under harsh environmental condition. Fig. 5 showed that all the bacterial strains were able to produce variable amount of IAA. Two strains; *P. agglomerans* KD6.2 *and R. terrigena* PCM8 were found best in IAA production (6.45 and 10.33 ppm.hour-1 respectively).

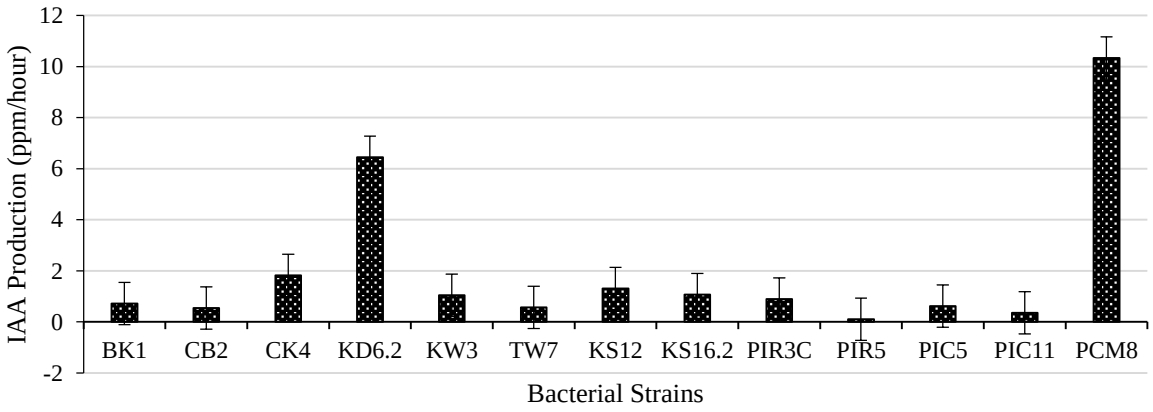

Figure 5. IAA production of ACC deaminase producing bacteria.

Most research showed that IAA producing oorganism are Gram negative [21]. Present study showed that the high IAA producing strains were Gram negative. IAA production by bacteria can vary among different species and strains, and it is also influenced by culture condition, growth stage and substrate availability. The property of synthesizing IAA is considered as effective tool for screening beneficial microorganisms suggesting that IAA producing bacteria have profound effect on plant growth [22].

3.2.3. Effect of the use of carbon sources for bacterial growth. Carbon (C) is one of the essential elements of nutrition and energy for the growth and activity of bacterial cells. Each bacterium has a different ability to use carbon for growth. Bacteria can utilize carbon sources from organic acids, amino

acids or carbohydrates such as glucose, fructose, cellulose, mannitol and so on [23]. The effect of using carbon sources (C) on bacterial growth on minimal medium (MM) is shown in Figure 6.

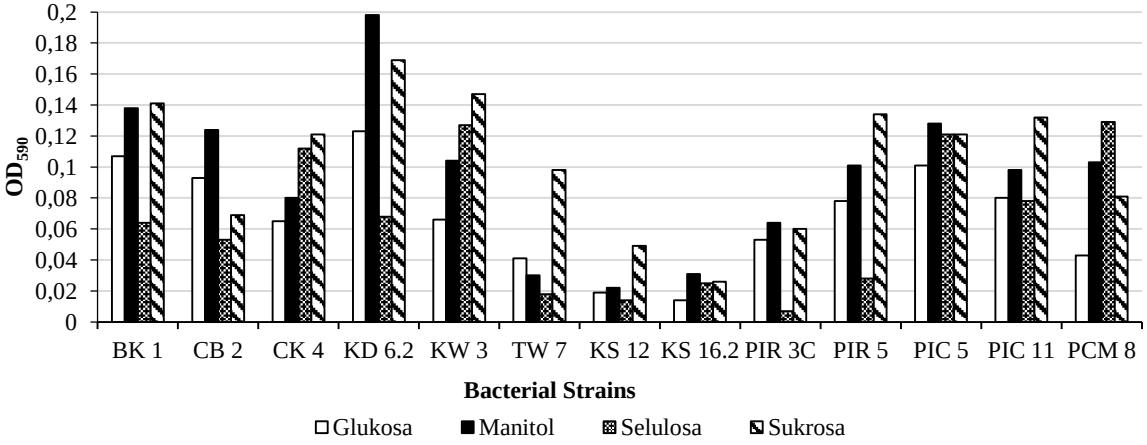

Figure 6. Effect of carbon sources on bacterial growth

Based on Figure 6, all bacterial strains are able to grow on the medium with the addition of several different carbon sources. The highest growth of bacteria on average occurs in the medium with the addition of carbon sources mannitol (KS16.2, PIC5, KD6.2, PIR3C, CB2) and sucrose (BK1, TW7, CK4, PIR5, KS12, KW3, PIC11), except PCM 8, the highest growth occurs in the medium with cellulose carbon sources. In general, microorganisms use short-chain carbon sources so they are easily metabolized in the growth process, one of which is glucose [24].

It was known that many strains of bacteria that use sugar alcohol (mannitol), sugar from the disaccharide group (sucrose) and polysaccharides (cellulose). The use of longer chain carbon sources for bacterial growth can occur due to the catabolite mechanism. The mechanism of catabolite carried out by bacteria is carbon catabolite repression (CCR). Repression of carbon catabolites allows bacteria to use preferred and easily decomposed carbon sources for the growth process [25] and [26].

3.2.4. Antagonistic activity. One indirect mechanism of PGPB is antagonism activity against pathogenic microorganisms. PGPB is able to inhibit the growth of pathogenic microorganisms by producing secondary metabolites [2]. Antagonistic activity of ACC deaminase-producing bacteria against pathogenic bacteria *X.oryzae pv. oryzae* and *R.solanacearum* are shown in Table 2.

X.oryzae pv.oryzae is a pathogenic bacterium that causes leaf blight on rice and significantly reduces crop yields by 50-60% [26], while *R.solanacearum* is one of the soil pathogenic bacteria that causes wilt disease of more than 250 species of plants around the world [27]. Several groups of bacteria are known to have antagonistic power to the bacterium *X.oryzae pv.oryzae* and *R.solanacearum*. The bacterial groups include *Bacillus*, *Pseudomonas* and *Streptomyces*. The mechanism is to produce antibiotic compounds (diffcidin, bacilysin, iturin), siredofor, HCN, and growth hormone (indole acetic acid / IAA) [28]; [29]; [30].

Based on Table 2, thirteen of ACC deaminase producing bacteria have no antagonistic activity against *X.oryzae pv oryzae* bacteria and *R.solanacearum* on the medium. This may be due the ACC deaminase producing bacteria does not produce secondary metabolites that can inhibit the growth of *X.oryzae pv oryzae* bacteria and *R.solanacearum*. According to [31], the formation of inhibitory zones against pathogens is influenced by the main secondary metabolites produced by bacteria, namely antibiotic compounds.

Table 2. Antagonistic activity of ACC deaminase-producing bacteria against pathogenic bacteria

No.	Bacterial strain	Pathogen bacteria	
		X.oryzae pv. *oryzae*	*R.solanacearum*
1.	*S. multivorum* BK1	-	-
2.	*B. mycoides* CB2	-	-
3.	*P. dispersa* CK4	-	-
4.	*P. agglomerans* KD6.2	-	-
5.	*E. ludwigii* KW3	-	-
6.	*B. aryabhattai* TW7	-	-
7.	*P. monteilii* KS12	-	-
8.	*P. putida* KS16.2	-	-
9.	*P. putida* PIR3C	-	-
10.	*S. maltophilia* PIR5	-	-
11.	*L. pakistanensis* PIC5	-	-
12.	*B. aryabhattai* PIC11	-	-
13.	*R. terrigena* PCM8	-	-

3.2.5.The effect of Cr^{6+} on bacterial growth. Heavy metal is one of the abiotic factors that influence the growth of microorganisms, one of which is Cr^{6+}. The effect of Cr^{6+} on the growth of ACC deaminase-producing bacteria is shown in Table 3. The thirteen strains of bacteria were able to grow on medium with Cr^{6+} concentration up to 300 mg.L-1, but an increase in Cr^{6+} concentration resulted in the growth of several bacterial strains decreased. This is likely due to the ability of bacteria to adapt to environments that contain heavy metals.

Table 3. Effect of Cr^{6+} concentration on the growth of ACC deaminase-producing bacteria.

No	Bacterial Strain	Concentration of Cr^{6+} ($mg.L^{-1}$)								
		0	5	25	50	100	150	200	250	300
1	*S. multivorum* BK1	+++	+++	+++	+++	+	+	+	+	+
2	*B. mycoides* CB2	+++	+++	+++	+++	+++	+++	+++	+++	+++
3	*P. dispersa* CK4	+++	+++	+++	+++	+++	++	++	++	+
4	*P. agglomerans* KD6.2	+++	+++	+++	+++	+++	+++	++	++	+
5	*E. ludwigii* KW3	+++	+++	+++	+++	+++	+++	+++	+++	+++
6	*B. aryabhattai* TW7	+++	+++	+++	+++	+++	+++	+++	+++	+++
7	*P. monteilii* KS12	+++	+++	+++	+++	+++	+++	++	++	+
8	*P. putida* KS16.2	+++	+++	+++	+++	+++	+++	+++	+++	+++
9	*P. putida* PIR3C	+++	+++	+++	+++	+++	+++	+++	+++	+++
10.	*S. maltophilia* PIR5	+++	+++	+++	+++	+++	+++	+++	+++	+++
11.	*L. pakistanensis* PIC5	+++	+++	+++	+++	+++	+++	+++	+++	+++
12.	*B. aryabhattai* PIC11	+++	+++	+++	+++	+++	+++	+++	+++	+++
13.	*R. terrigena* PCM8	+++	+++	+++	+++	+++	+++	+++	+++	+++

According to [32], some microorganisms can adapt to environments with heavy metal stresses. Adaptation of microorganisms to heavy metal stress is by forming a defense mechanism. Some of the

defense mechanisms carried out include absorption, mineralization, metal absorption and accumulation; extracellular precipitation and oxidation or enzymatic reduction; and cell efflux.

4. Conclusion

Thirteen of ACC deaminase producing bacteria were isolated from root tissue of 13 different plant sample and 13 different locations at Special Region of Yogyakarta. The ACC deaminase producing bacteria varied in their phosphate solubilizing activity, IAA production, and antagonistic activity against the bacterial pathogen *X. oryzae pv. oryzae* and *R solanacearum*. The optimum growth of 13 strains of ACCD-producing bacteria occurs in the range of pH 6-8, temperature 25-30˚C, and bacteria are aerobic to facultative anaerobic. All bacterial strains can use glucose, sucrose, cellulose and mannitol as carbon and energy sources. The bacterial strain has a tolerance to Cr^{6+} between 100-300 mg. L-1. This study can provide basis for the large scale isolation and utilization as biofertilizer of more efficient PGPB for commercial production of inoculum.

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SUPPLEMENTARY INFORMATION
Additional Supporting Information

Table S1. Phenotypic characterization of bacterial strains based on their cell morphology and gram staining. The cell morphology was examined microscopically with 1000x magnification. The Gram character was determined by gram staining method.

No.	Bacterial Strains	Margin of Colony	Colony Shape	Cell Shape	Gram Staining
1	*Sphingobacterium multivorum* BK1	*Entire*	*Round*	Rod	Negative
2	*Bacillus mycoides* CB2	*Filiform*	*Filamentous*	Rod	Positive
3	*Pantoea dispersa* CK4	*Entire*	*Round*	Rod	Negative
4	*Pantoea agglomerans* KD6.2	*Curled*	*Round*	Rod	Negative
5	*Enterobacter ludwigii* KW3	*Entire*	*Round*	Rod	Negative
6	*Bacillus aryabhattai* TW 7	*Crenate*	*Circulair*	Rod	Positive
7	*Pseudomonas monteilii* KS 12	*Undulate*	*Circulair*	Rod	Negative
8	*Pseudomonas putida* KS 16.2	*Lobate*	*Circulair*	Rod	Negative
9	*Pseudomonas monteilii* PIR 3C	Undulate	*Circulair*	Rod	Negative
10	*Stenotrophomonas maltophilia* PIR 5	Entire	*Circulair*	Rod	Negative
11	*Lysinibacillus pakistanensis* PIC 5	Crenate	*Circulair*	Rod	Positive
12	*Bacillus aryabhattai* PIC 11	Crenate	*Circulair*	Rod	Positive
13	*Raoultella terrigena* PCM 8	Lobate	*Circulair*	Rod	Negative