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Growth Enhancement and Disease Reduction of Soybean by 1-Aminocyclopropane-1-Carboxylate Deaminase-Producing *Pseudomonas*

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Abstract: Problem statement: 1-Aminocyclopropane-1-Carboxylate (ACC) deaminase-producing bacteria have been known to ameliorate the inhibition-effect of increase concentration of ethylene in higher plants, which can be triggered by high concentration of indole-3-Acetic Acid (IAA) and/or by the presence of plant pathogens. This study examined the potential use of *Pseudomonas* isolates producing ACC deaminase as well as IAA to enhance soybean growth and reduce disease incidence in soil containing pathogenic fungi. **Approach:** Eleven promising ACC deaminase-producing isolates of Pseudomonas were retested in vitro for their ACC deaminase activity and IAA production and evaluated their potential antagonist against root-nodule bacteria. Non antagonist isolates were further tested for their ability to enhance soybean growth and reduce disease incidence in sterile and non-sterile soils containing root-pathogenic fungi *Fusarium oxysporum*, *Sclerotium rolfsii* and *Rhizoctonia solani*. **Results:** All isolates produced ACC deaminase as well as IAA, but 3 out of 11 isolates inhibited at least one strain of rhizobia which limit their use for soybean. The isolates increased some aspects of soybean growth, but most of the increases were not significantly different from untreated control. Most isolates significantly increased the survival rates of soybean in soil containing pathogenic fungi although their ability to reduce plant weight loss varied across pathogen treatments. **Conclusion:** The ability of *Pseudomonas* producing ACC deaminase as well as IAA to increase plant growth was less significant than that of the isolates to reduce disease incidence. The higher the destructive effect of the pathogens, the better was the ability of the isolates to reduce the disease.

Key words: ACC deaminase, disease incidence, *Fusarium oxysporum*, growth enhancement, IAA, *Pseudomonas*, *Rhizoctonia solani*, *Sclerotium rolfsii*, soybean

INTRODUCTION

A number of 1-Aminocyclopropane-1-Carboxylate (ACC) deaminase-producing bacteria have been known to help plant growth by ameliorating the negative effects of increased concentration of ethylene in higher plants. As a senescing hormone (Abeles *et al*., 1992; Arshad and Frankenberger, 1991), increased concentration of ethylene after seed germination inhibits root development (Glick, 1995; Shah *et al*., 1998) and nodulation of various legumes (Ma *et al*., 2003). Regarding plant-pathogen interactions, ethylene produced during plant infection promotes disease rather than alleviating it (Van Loon *et al*., 2006). Ample studies on the benefits of ACC deaminase-producing bacteria in reducing ethylene synthesis caused by various biotic and abiotic stresses have been reported, such as those caused by high concentration of IAA (Mayak *et al*., 1999), water logging (Grichko and Glick, 2001), nutritional stress (Belimov *et al*., 2002), drought (Mayak *et al*., 2004), organic and inorganic pollutants (Reed and Glick, 2005; Belimov *et al*. 2001), high salts (Saravanakumar and Samiyappan, 2007) and pathogenic infection (Wang *et al*., 2000; Dey *et al*., 2004; Shaharoona *et al*., 2006). Thus, applying ACC deaminase producing bacteria to reduce ethylene synthesis in the plant, whenever it starts accelerating may become one of the promising strategies to increase plant growth and prevent disease development.

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Our previous experiments on the promoting effects of *Pseudomonas* producing Indole-3-Acetic Acid (IAA) on soybean seedlings were also related to their ACC deaminase activities. Their ACC deaminase activities optimized the promoting effects of IAA and increased root elongation and shoot weight of soybean seedlings (Husen *et al*., 2009). It suggests that the bacteria may have the capacity to reduce disease development as described by previous reports (Wang *et al*., 2000; Glick *et al*., 2007). However, since the effectiveness of the bacteria may vary depending on the nutrient status of the media (Shaharoona *et al*., 2006; 2007) and the fitness of the bacteria with host plants (Silvia *et al*., 2004), these abilities are worth to be verified.

The objective of the present study was to examine the ability of *Pseudomonas* producing ACC deaminase as well as IAA to enhance soybean growth and reduce disease development in potted soil containing pathogenic fungi.

MATERIALS AND METHODS

Bacterial isolates, pathogenic fungi and growth media: Eleven isolates of plant growth promoting-*Pseudomonas*, designated as Crb5, Crb12, Crb17, Crb24, Crb26, Crb46, Crb47, Crb49, Crb53, Crb56 and Crb94 were used in the study. The origin of the bacteria was from the rhizosphere of soybean grown in Plumbon agricultural area in Cirebon, West Java, Indonesia. The bacteria were selected from 81 indole-3-acetic acid-producing *Pseudomonas* as well as 1-*aminocyclopropane*-1-*carboxylate* (ACC) deaminase (E.C.4.1.99.4) and significantly promoted soybean seedlings under growth chamber conditions (Husen *et al*., 2009). Two rhizobial strains for inhibition tests, i.e., *Bradyrhizobium japonicum* Bj11 and *Sinorhizobium fredii* Rif5 and three known phytopathogenic fungi for disease reduction tests, i.e., *Fusarium oxysporum*, *Sclerotium rolfsii* and *Rhizoctonia solani* were obtained from the Laboratory of Soil Biology, Indonesian Soil Research Institute (ISRI); Laboratory of Microbiology, Department of Biology, Faculty of Mathematics and Natural Sciences, Bogor Agricultural University (IPB); and the culture collection of Department of Plant Protection, Faculty of Agriculture, IPB. Seeds of soybean (*Glycine max* L. Merr.) cv. Wilis were obtained from the Indonesian Center for Biotechnology and Genetics Resources Research and Development, Bogor.

Depending on the assay, each isolate from stock culture was grown in Dworkin-Foster (DF) Minimal Salts (MS) medium (Dworkin and Foster, 1958) supplemented with either ACC or ammonium sulfate to check the activity of ACC deaminase, MS tryptophan medium (Frankenberger and Poth, 1988) for IAA tests, King's B Medium (KBM) for antagonism tests and in M26 rich medium for bacterial cell production. The rhizobial strain was also grown in KBM for inhibition tests, while the pathogenic fungus was cultured and maintained in potato dextrose broth. The composition of DF MS medium was 4 g KH_2PO_4, 6 g Na_2HPO_4, 0.2 g $MgSO_4.7H_2O$, 1 mg $FeSO_4.7H_2O$, 10 µg H_3BO_3, 10 µg $MnSO_4$, 70 µg $ZnSO_4$, 50 µg $CuSO_4$, 10 µg MoO_3, 2 g glucose, 2 g gluconic acid, 2 g citric acid, 1000 ml distilled water+either 0.3033 g L^{-1} ACC or 2 g L^{-1} ammonium sulfate and solidified with 15 g agar. The MS-tryptophan medium was a mixture of 900 ml MS medium and 100 ml stock solution of L-tryptophan solidified with 15 g agar. The MS medium contained (in 900 ml distilled water) 1.36 g KH_2PO_4, 2.13 g Na_2HPO_4, 0.2 g $MgSO_4.7H_2O$ and trace elements and stock solution of L-tryptophan contained (in 100 ml distilled water) 10 g glucose, 1 g L-tryptophan and 0.1 g yeast extract. The KBM contained 20 g peptone, 10 ml glycerol, 1.5 g K_2HPO_4, 1.5 g $MgSO_4.7H_2O$, 15 g agar (for solid media) and 1000 ml distilled water. The M26 broth medium contained 10 g beef extract, 10 g proteose peptone, 5 g NaCl and 1000 ml distilled water. Unless otherwise noted, all media constituents were mixed and sterilized by autoclaving at 121°C for 15 min. The heat-labile ACC and stock solution of L-tryptophan was filtered-sterilized each by 0.2 µm membrane filter before adding to the sterilized medium.

A bulk of the Ap horizon of Ultisols for pot experiments was obtained from upland agricultural area in Jasinga, Bogor. Prior to potting, the soil was prepared by passing through a 2-mm sieve and analyzed for soil texture, pH, C-organic and selected nutrients according to the standard procedures of the Soil Chemistry Laboratory of Indonesian Soil Research Institute (ISRI) in Bogor. Data on soil sample analyses in Table 1 showed that the soil is acidic with fine texture, low organic content and low fertility status.

***In vitro* tests:** The ACC deaminase activity of each isolate was verified by growing in the DF salt minimal medium, supplemented with either ACC (DF-ACC) or ammonium sulfate (DF-Ammonium Sulfate) as described by Glick *et al*. (1995). The DF-Ammonium Sulfate or DF-ACC salts minimal medium was used to check whether the ACC deaminase produced by the isolates was part of a constitutive or inducible system as proposed by Jacobson *et al*. (1994).

Table 1: Texture and some chemical characteristic of Ultisols from Jasinga, Bogor, Indonesia

					(Extracted HCl 25%)			
	Texture	pH	C-org.	Total N	P_20_5	K_2O	CEC	BS
Unit	-	-	-------- g/kg -------		---------- mg/kg ---------		cmol $_{(+)}$/kg	%
Value	SiCL	4.5	17	1.3	300	110	13.67	40
Status	Fine	Acidic	Low	Low	Medium	Low	Low	Low

CEC = Cation Exchange Capacity; BS = Base Saturation; SiCL = Silty Clay Loam

The DF salts minimal medium without any supplements was included in the tests to check whether the isolates were able to fix atmospheric dinitrogen. Each isolate was grown in M26 broth culture for 24 h with constant shaking at 125 rev min^{-1} at room temperature (± 28°C). After overnight growth, the bacterial suspension was centrifuged at 4000 *g* for 10 min and cell pellets were washed with sterile distilled water. Diluted cell pellets were then spread onto each agar plate containing DF-ACC, DF-Ammonium Sulfate, or DF salts minimal media. The growth of bacterial colonies on solid media after 48 h incubation was used as the confirmation of the traits.

The ability of the isolates to produce IAA was checked using ferric chloride-perchloric acid reagent ($FeCl_3$-$HClO_4$) adapted from Gordon and Weber (1951). The cell pellets of each isolate were prepared as described above and then spread onto agar plates of MS-tryptophan and incubated for 48 h in the dark. Subsequently bacterial colonies on agar plates were covered with $FeCl_3$-$HClO_4$ reagent. Red color around bacterial colonies was used as the confirmation of positive IAA production.

The potential antagonism of each *Pseudomonas* isolate against root nodule rhizobia of *Bradyrhizobium japonicum* Bj11 or *Sinorhizobium fredii* Rif5 (test bacteria) was tested by dual culture detection technique adapted from Weaver and Mickelson (1994). The ability of each potential antagonist to inhibit the growth of test bacteria was conducted in KBM agar plates inoculated with a lawn of bacterial cells from each of the eleven isolates. A 100 µL of a suspension of the isolate containing approximately 10^7 cells mL^{-1} (from 24 h broth culture) was spread onto the surface of KBM agar plates. A 100 µl drop of a suspension of each of *B. japonicum* Bj11 or *S. fredii* Rif5 (from late-logarithmic growth phase) was applied to the center of agar plates. A plate without isolate inoculation was used as the control. All inoculated plates were incubated at room temperature for 5 days. The circle inhibition indicated by clear halos surrounding rhizobial colonies was considered positive for antagonism. Bacterial isolates with positive of production of ACC deaminase as well as IAA, which did not show antagonist against rhizobia, were selected for greenhouse experiments.

Growth enhancement assay: The test was conducted in green house conditions using sterile and non-sterile sieved soil in plastic pots following the protocol described by Cattelan *et al*. (1999). A non-sterile pot contained about 2 kg of sieved soil, while a sterile pot filled with a mixture of 400 g of sieved soil+100 g quart sand autoclaved twice, on 2 consecutive days (one h per day).

Plastic pots were surface disinfected with 95% alcohol. Water was added daily to each potted soil to attain a water-content equivalent to field capacity (~ -0.03 MPa). Each experiment was performed in a randomized complete block design with 5 replications.

Bacterial inoculants for the experiments (selected from previous tests) were prepared by growing in KBM broth and subsequently transferred to M26 rich medium to produce high numbers of bacterial cells. After overnight growth by constant shaking at 125 rev min^{-1} at room temperature, bacterial cells were centrifuged at 4000 *g* for 10 min. The cell pellets were washed with 100 mM $MgSO_4$ and then re-suspended in 100 mM $MgSO_4$. Prior to being used for seed inoculation, the absorbance of the cell suspension was adjusted to about 0.5 at 780 nm using a UV Spectrophotometer, which was equal to 108-109 cells mL^{-1}. Soybean seeds, similar in size, were surface disinfected by soaking in 70% alcohol for 1 minute and 1% sodium hypochlorite for 5 min and then rinsed with sterile distilled water several times. The seeds were immersed in either 100 mM $MgSO_4$ which acted as a blank control (untreated), or a suspension of bacterial cells in 100 mM $MgSO_4$, for 1 h. The treated and untreated seeds were first germinated in sterile Petri dishes containing double wet filter papers. Two germinated seeds with radicle of about 1 cm from the same treatment were planted in each pot. After emergence, plants were thinned to one plant per pot and grown for 14 days. At harvest, the shoots were cut off and the roots were washed and their fresh weight was recorded.

Disease reduction assay. The sterile and non-sterile potted soils were prepared as described above, except the water-content of the soils was adjusted to be moister (equivalent to -0.01 MPa) to allow the development of added fungal pathogens. The tests in each sterile or non-sterile soil were conducted in three separate experiments (three kinds of pathogenic fungi) following

the protocol described by Wang *et al*. (2000) and Cattelan *et al*. (1999). Each pathogen, i.e., *Fusarium oxysporum*, *Sclerotium rolfsii* or *Rhizoctonia solani*, was grown in potato dextrose broth with constant shaking at room temperature and harvested after 4 and 5 days. Before inoculation to the soil, the harvested fungus was separated with mixer and suspended in sterile distilled water for 1 h. The fungal suspension was inoculated to the potted soil and the fungal density was determined by serially plating a portion of the solution. The final counts of *F. oxysporum*, *S rolfsii* and *R solani* per gram of soil were about 8.1×10^4, 1.2×10^3, 1×10^3 colony-forming units (cfu g^{-1}), respectively. In experiments with sterile soil, besides inoculation of fungal pathogens, the sterile potted soil was also inoculated with bacterial isolates (prepared as described above) at the density ~10^7 cfu g^{-1} following the protocol described by Wang *et al*. (2000). Soybean seeds preparation and treatment with bacterial isolates were conducted as described above. In all experiments, the treatments included non-inoculated soil with fungal pathogen (and with bacterial inoculants) planted with untreated soybean seeds (untreated control) as well as inoculated soil with fungal pathogen planted with untreated soybean seeds.

The experiments using sterile soils were set in a randomized completely block design. Each treatment was assigned to 3 replicates where each replicate consisted of 4 pots and each pot contained 4 plants. In non-sterile soils, the experiments were conducted in a completely randomized design and each treatment was with 5 replications. Plants were grown for 14 days. Disease reduction was evaluated by the number of plants surviving and fresh weight loss. Infected plants were considered dead when they failed to recover within 14 days.

Statistical analysis all data in each experiment were analyzed by Analysis Of Variance (ANOVA) and treatment means were separated by the Duncan Multiple Range Test (DMRT) using the SAS systems for Windows 6.12.

RESULTS

Results on *in vitro* assay are presented on Table 2. All isolates were able to grow in DF-ACC and DF-ammonium sulphate minimal salts medium and use ACC or NH_4SO_4 as their sole source of N as an indication of their ACC deaminase activity. However, none of the isolates was able to grow in DF minimal salts medium without any addition of N sources indicating that the isolates did not have ability to fix atmospheric nitrogen. All isolates were able to produce IAA in minimal salt medium supplemented with tryptophan (exhibited by the red color around bacterial colonies with $FeCl_3$-$HClO_4$ reagent). With regard to rhizobial inhibition, 3 isolates (26, 53 and Crb94) inhibited at least one strain of rhizobia (*Bradyrhizobium japonicum* Bj11 and/or *Sinorhizobium fredii* Rif5); thereby limiting their use for soybean (Table 2). Based on the results, only 8 out of 11 isolates were potentially used as plant growth promoting bacteria for soybean.

Growth responses of soybean treated with 8 selected isolates are presented in Table 3. The isolates increased some aspects of plant growth in comparison to the untreated plants. The significant increase of plant fresh weight was exhibited by those treated with *Pseudomonas* 5, 24, 46 and Crb56 in sterile soils and with 12, 17 and Crb24 in non-sterile soils.

The ability of the isolates to reduce soybean root diseases is presented in Table 4. Plants infected with *Fusarium oxysporum*, *Sclerotium rolfsii* and *Rhizoctonia solani* showed yellow to yellowish leaves, brownish leaves followed by softened or rotten stems and stunted plant growth, respectively. The survival rates of untreated soybean in soils containing *F. oxysporum*, *S. rolfsii* and *R. solani* were about 85, 69 and 58%, respectively, indicating that the destructive effects of the former pathogen were less than the later two pathogens. In sterile soils, most isolates increased the survival rates of soybean although plant fresh weight losses were variable, which could be due to different time recovery of infected plants across the treatments.

Table 2: ACC deaminase activity, IAA production, phenotypic characteristics of *Pseudomonas* isolates (Crb) used in the study

					Antagonism against[¶]	
Isolates	DF-ACC[†]	DF-Ammonium Sulfate[‡]	DF	IAA[§]	Bj11	Rif5
Crb5	+	+	-	+	-	-
Crb12	+	+	-	+	-	-
Crb17	+	+	-	+	-	-
Crb24	+	+	-	+	-	-
Crb26	+	+	-	+	+	-
Crb46	+	+	-	+	-	-
Crb47	+	+	-	+	-	-
Crb49	+	+	-	+	-	-
Crb53	+	+	-	+	-	+
Crb56	+	+	-	+	-	-
Crb94	+	+	-	+	+	+

[†]: Dworkin-Foster minimal salt medium (DF) supplemented with ACC; [‡]: DF supplemented with NH_4SO_4; [§]: IAA (indole-3-acetic acid) production; [¶]: Antagonism against *Bradyrhizobium japonicum* Bj11 and *Sinorhizobium fredii* Rif5

Table 3: Effects of ACC deaminase-producing *Pseudomonas* (Crb) on soybean growth in sterile and non-sterile soils under greenhouse conditions

	Sterile soil		Non-sterile soil	
Treatment	Shoot height (cm)	Total fresh weight (g)	Shoot height (cm)	Total fresh weight (g)
Untreated	24.7a	2.66c	24.1	2.97cd
Crb5	24.8a	3.47a	25.4abc	3.06bcd
Crb12	22.8b	2.67c	25.3abc	3.38a
Crb17	23.3ab	2.68c	25.3abc	3.27ab
Crb24	24.1ab	2.94b	26.5a	3.31a
Crb46	24.2ab	2.95b	25.0abc	2.88d
Crb47	24.6a	2.70c	26.2ab	3.03cd
Crb49	23.9ab	2.63c	24.7bc	2.95d
Crb56	24.4ab	2.94b	24.0c	3.19abc

Numbers within a column followed by the same letter are not significantly different at 5% level by DMRT. Values are means from five replications

Table 4: Reduction in severity of diseases caused by pathogenic fungi measured by surviving plants and plant fresh weight following inoculation with ACC deaminase-producing *Pseudomonas* in sterile and non-sterile soils under greenhouse conditions

	Sterile soil			
Treatment[†]	Average number of surviving plants[§]	Survival rate (%)	Sterile soil Total fresh weight (g)[§]	Non-sterile soil Total fresh weight (g)[¶]
Untreated	4.00a	100	9.98a	2.54ab
Crb5+Fo	4.00a	100	7.95cd	2.14c
Crb12+Fo	3.92a	98	8.12bcd	2.48b
Crb17+Fo	3.92a	98	7.59de	2.53ab
Crb24+Fo	3.83a	96	8.73b	2.71a
Crb46+Fo	3.75ab	94	7.05e	2.59ab
Crb47+Fo	4.00a	100	8.45bc	2.24c
Crb49+Fo	3.83a	96	6.28f	2.29c
Crb56+Fo	3.92a	98	7.65de	2.26c
Fo	3.42b	85	7.52de	2.25c
Untreated	4.00a	100	8.01a	2.22a
Crb5+Sr	3.42ab	85	6.77b	0.42f
Crb12+Sr	3.75ab	94	7.94a	0.85d
Crb17+Sr	3.25bc	81	6.51b	1.61b
Crb24+Sr	3.33bc	83	6.58b	1.10c
Crb46+Sr	3.50ab	88	5.86c	1.57b
Crb47+Sr	3.33bc	83	6.88b	0.67e
Crb49+Sr	3.67ab	92	6.90b	0.21g
Crb56+Sr	3.50ab	88	6.96b	0.33f
Sr	2.75c	69	6.53b	0.15g
Untreated	4.00a	100	8.74a	1.64a
Crb5+Rs	3.83ab	96	6.82b	1.27c
Crb12+Rs	3.42bc	85	5.15a	1.14c
Crb17+Rs	3.17cd	79	4.77b	1.24c
Crb24+Rs	2.75de	69	4.44b	1.24c
Crb46+Rs	3.42bc	85	5.13c	1.20c
Crb47+Rs	3.17cd	79	5.37b	1.17c
Crb49+Rs	3.42bc	85	5.85b	1.45b
Crb56+Rs	3.58abc	90	6.16b	1.75a
Rs	2.33e	58	4.31b	0.67d

[†]: Pathogenic fungi of *Fusarium oxysporum* (Fo), *Sclerotium rolfsii* (Sr) or *Rhizoctonia solani* (Rs) and *Pseudomonas* (Crb) were added as described in Materials and Methods, an uninoculated control (untreated) and a treatment with only the pathogen (Fo, Sr, or Rs) were also included. [§]: Numbers within a column in each set of the experiments followed by the same letter are not significantly different at 5% level by DMRT. Values are means from three replications, each replication has four pots, and each pot has four plants. [¶]: Numbers within a column in each set of the experiments followed by the same letter are not significantly different at 5% level by DMRT. Values are means from five replications

The significant reduction of plant weight loss was shown by plants treated with isolates Crb24 and Crb47; Crb12; and all isolates, except Crb24 in soils containing *F. oxysporum*, *S. rolfsii* and *R. solani*, respectively. On the other hand, in non-sterile soils, most plants treated with the isolates significantly reduced plant weight loss, especially in soils containing *S. rolfsii* and *R. solani*.

DISCUSSION

The importance of ACC deaminase-producing bacteria on plant growth is to reduce ethylene synthesis in plant tissues which is commonly triggered by a number of biotic and abiotic factors (Glick *et al*., 2007), including by increased concentration of IAA. ACC

deaminase-producing bacteria degrade ACC (ethylene precursor) into ammonia and α-ketobutyrate as their source of nitrogen and carbon (Jacobson *et al*., 1994; Glick *et al*., 1998); thereby ameliorating the inhibition-effects of ethylene. In the present study, IAA produced by the isolates may promote soybean growth without being offset by increased synthesis of ACC (triggered by IAA) since their ACC deaminase activity can degrade it and prevent ethylene synthesis. However, growth promotion of soybean by the isolates was not optimum (Table 3). Low nutrient status of the soils used in the study could affect their efficacy in growth promotion. Shaharoona *et al*. (2006; 2008) reported that plant growth promotion of bacteria containing ACC deaminase occurred when optimum levels of N fertilizer were applied. The results suggest that application of the appropriate doses of fertilizers into soil with low fertility status is required to get maximum benefit in utilizing these bacteria.

Regarding plant-pathogen interactions, it has been known that several plant pathogenic microbes have developed the ability to modulate signaling processes mediated by plant hormones as a strategy for manipulating plant growth or host physiology (Lund *et al*., 1998; Van Loon *et al*., 2006). Plants with a high level of ethylene will be in a state of ethylene-stress which inhibits plant growth. Increasing levels of ethylene exacerbates the stress and weakens plant protection against the diseases. The presence of ACC deaminase-producing bacteria residing in the root surface area reduces ethylene synthesis by hydrolyzing ACC exuded by the root and taken up by the bacteria as their source of nitrogen. Lowering ethylene levels in plants through the action of ACC deaminase-producing bacteria ameliorate the inhibitory effects of ethylene. In the present study the ability of the isolates to reduce soybean diseases was exhibited by most of the isolates although some isolates behaved differently in sterile and non-sterile soil conditions as well as the kinds of the pathogens. This variable effect suggests that the efficacy of the isolates in ameliorating disease were influenced by soil conditions and severity of pathogens, or otherwise their efficacy is specific to a certain pathogen. In line with the results, a previous report by Glick *et al*. (2007) concludes that the efficacy of ACC deaminase-producing bacteria on plant growth may not be clearly observed at stress-free conditions.

CONCLUSION

This study confirms the previous works on the benefits of ACC deaminase-producing bacteria on plant growth. However, the beneficial effects of *Pseudomonas* producing ACC deaminase as well as IAA on soybean growth may vary depending on growth medium. The isolates increased some aspects of soybean growth and reduced disease incidence caused by *Fusarium oxysporum*, *Sclerotium rolfsii* and *Rhizoctonia solani*. The ability of the isolates to increase plant growth was less effective than that to reduce disease incidence. The higher the destructive effect of the pathogens, the better was the ability of the isolates to reduce the disease. Further tests are worth to evaluate whether or not the fertility status of the soil and the severity of pathogens influence the efficacy.

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