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Exploitation of PGPR Endophytic *Burkholderia* Isolates to Enhance Organic Agriculture

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Abstract: Although many bacterial species have been isolated from the rhizosphere of various crop plants, the recent discovery is *Burkholderia* sp., an endophytic bacterium. In this study, the *Burkholderia* isolates *viz*., RB_1 (Rice *Burkholderia* 1), MB_2 (Maize *Burkholderia* 2), SB_3 (Sugarcane *Burkholderia* 3) and BB_4 (Black gram *Burkholderia* 4) were enumerated from the root, stem and leaf samples of four different crops *viz*., rice, maize, sugarcane and black gram using N-free BAz (*Burkholderia* Azelaic acid) medium, in which black gram roots were observed higher population. Further, growth promoting activities of the *Burkholderia* isolates were examined, the maximum production of IAA and GA was noticed on the BB_4 as compared to other isolates and the cytokinin production was recorded more in isolates SB_3 followed by BB_4. Among the four isolates, maximum amount of salicylate type was noticed in RB_1 and catechol type was recorded higher in BB_4 which showed that these isolates were capable to produce Siderophore. The ACC deaminase activity of the isolates were exhibited, the BB_4 was recorded more followed by SB_3. Therefore, the endophytic *Burkholderia* isolates also the important contributor to the crop growth through secretion of growth promoting substances, production of siderophore and ACC deaminase activities may improve the Agriculture production.

Keywords: *Burkholderia* Isolates, Endophytes, Indole Acetic Acid, Gibberellic Acid, Cytokinin, Siderophore, ACC Deaminase

1. Introduction

It has been well established that rhizosphere region forms a conducive environment for various microorganisms and always witnessed with enhanced activity when compared to non-rhizosphere region [1, 2]. The studies demonstrated the role of different microbes of rhizosphere region in particular plant growth promoting rhizobacteria has been well elucidated. Beneficial rhizobacteria that stimulate plant growth are usually referred to as Plant Growth Promoting Rhizobacteria or PGPR group [3, 4] that includes different bacterial species and strains belonging to genera such as *Acetobacter, Azospirillum, Azotobacter, Bacillus, Burkholderia, Herbaspirillum* and *Pseudomonas* [5-7]. Several studies have been proposed explaining the potential of PGPR on various agricultural crops which includes phosphorus solublisation [8], growth promotion [9], plant pathogen control [10], micronutrients solubilisation [11], nutrient recycling [11] and so on. Further in order to establish positive interactions with roots of the plants, production of PGPR organism in larger scale has been initiated and following which many different PGPR organisms have been discovered and proposed by scientist.

Among PGPR organisms *Burkholderia cepacia* (previously known as *Pseudomonas*) a nutritionally versatile and gram-negative organism was first demonstrated in 1949 by Walter Burkholder of Cornel University. But he proposed it as the phytopathogen responsible for bacterial rot of onions [12] which forms

endophytic association with the plants. Though it has been introduced as phytopathogen, further studies on the strains confirmed its plant growth promoting properties. From then, interestingly *Burkholderia* sp. has been isolated from rhizosphere regions as well as different parts of plants. To state few, *H. seropedicae* and *Burkholderia* strains found to colonise the roots and shoots of the rice plants grown in JNFB and JMV semi-solid media in high numbers. High population of endophytic bacteria (10^5-10^8) were also observed in 25 rice genotypes grown on Philippines, but only in four genotypes was the incidence of endophytic diazotrophs [13]. The same has been reported by [14] with sugarcane. The cluster formed by the diazotrophic species *B. kururiensis* and *B. tuberum* was clearly different from the diazotroph *B. vietnamiensis* which belongs to the "*B. cepacia* complex" [15]. Another diazotrophic bacteria representative of the genus *Burkholderia* have been isolated from rice plants and sugarcane plants and named as *B. brasilensis* [14]. It also suggested that rice stems are more suitable niches for N_2 fixing endophytes than roots because more photosynthates are available to the bacteria [13].

Apart from rice and sugarcane the occurrence of *Burkholderia* sp. has been reported by many workers in Horticulture crops as well. Tentatively named species of *Burkholderia* recovered from various parts of banana and pineapple plants as *B. tropicalis* and *B. Brasilensis* [16]. Recently analysis of maize, sorghum and coffee plants grown under field conditions revealed the presence of the genus *Burkholderia*.

Apart from its ubiquitous association with several crops, there are several reports also stated the plant growth promoting activities of *Burkholderia* sp. to the host plant. Under diverse environmental conditions they are able to communicate and interact with the plants more efficiently than rhizospheric bacteria and significantly impact plant growth by enhancing nutrient uptake by host plants, produce several active compounds, suppresses pathogens by producing antibiotics, siderophores and antifungal compounds [17-21].

With respect to 1-aminocyclopropane-1-carboxylic acid (ACC) deaminase activity of growth of N_2 fixing *Burkholderia unamae* isolates using 1-aminocyclopropane-1-carboxylic acid (ACC) as the sole nitrogen source was tested on BAz-medium [22]. They have mechanism to produce the enzyme 1-aminocyclopropane-1-carboxylate (ACC) deaminase. The enzyme is responsible for the cleavage of the plant ethylene precursor, ACC, into ammonia and ketobutyrate [23]. It works on the principle by decreasing ethylene levels in plants which prevents plant growth inhibition.

Thus plants growing with these organisms with ACC deaminase activity will have longer roots and shoots. In addition other vital functions such as seed germination, root hair development, adventitious root formation, nodulation, leaf and fruit abscission, and flower and leaf senescence have been found to be influenced by ethylene [24, 25]. Hence, the organisms will have a positive influence on that too. In our earlier study *Burkholderia* isolates were collected from the root, stem and leaf samples of four different crops *viz.,* rice, maize, sugarcane and black gram, using N-free BAz (*Burkholderia* Azelaic acid) semi solid medium for isolation, screening of *Burkholderia* isolates *viz.,* RB_1 (Rice *Burkholderia* 1), MB_2 (Maize *Burkholderia* 2), SB_3 (Sugarcane *Burkholderia* 3) and BB_4 (Black gram *Burkholderia* 4) and their nitrogen fixing ability has been observed [26]. The study was also initiated by using them as Bioinoculant. In current study, *Burkholderia* isolates were enumerated and evaluated on plant growth promoting activities, production of siderophore and ACC deaminase activity.

2. Materials and Methods

2.1. Reference Strains

The reference strains of *Burkholderia* sp. *viz., Burkholderia vietnamiensis* and *Burkholderia tropicalis* were obtained from Prof. P. Vandamme, Gent University, Belgium and Dr. J. Balandreau, Institute of Research for Development, South Africa respectively.

2.2. Enumeration of Burkholderia sp. from Different Crop Plants

The method of enumeration of endophytes described by [27] was adopted for the isolation of *Burkholderia* from the root, stem and leaves of plant samples collected. The fresh plant samples were carefully separated into stem, roots and leaves. Each part was cut in to sections of 2-3 cm lengths and dried on absorbent towels. One gram of each part was taken and was surface sterilized for l min with 70 per cent ethyl alcohol and 1 per cent chloramines-T. The plant parts were washed thoroughly with sterile distilled water thrice to remove the traces of chloramines-T and homogenized in a sterile pestle and mortar in laminar chamber. The homogenized suspension was diluted to 10^{-3} and one ml of the suspension was poured on the plate containing BAz agar medium. The plates were incubated at room temperature for 3 days. The colonies were selected based on the morphological characteristic such as colour, shape, margin, elevation and growth pattern. The isolates were numbered accordingly and thereafter the culture was maintained in respective agar slants. After subsequent sub culturing the isolates were used for further studies.

2.3. Determination of Indole Acetic Acid Production

BAz broth was dispensed in 100 ml quantities in 250 ml flask and sterilized. Freshly filter sterilized solution of 0.2% L-Tryptophan was added to one set of flask. The media prepared without L-tryptophan was maintained as control [28]. One ml standard inoculum of *Burkholderia* isolates were added into each flask and incubated at 37°C for 7 days in dark. After 7 days of incubation, the culture was centrifuged at 6000 rpm to remove the bacterial cells. The

supernatant solution after centrifugation was adjusted to pH 2.8 with 1N HCl. Acidified supernatant of 15 ml volume was taken in 100 ml conical flask and equal volumes of diethyl ether was added to it and incubated in dark for 4 hrs. IAA extraction was done at 4°C by keeping overnight in a separating funnel using diethyl ether. The organic phase was discarded and the solvent phase was pooled and evaporated to dryness. To the dried residue 2 ml of methanol was added and the IAA present in the methanol extract was determined using the method described by [29]. To 0.5 ml of the above methanol extract 1.5 ml of distilled water and 4 ml of Salpers reagent (1ml of 0.5 M $FeCl_3$ in 50 ml of 35% Perchloric acid) were added and incubated in dark for one hour. The intensity of pink colour was read at 535 nm in a double beam spectrophotometer. From the standard curve prepared with known concentration of IAA, the quantity in the culture filtrate was determined and expressed as μg ml^{-1}.

2.4. Determination of Gibberellic Acid Production

2.4.1. Extraction of Gibberellins

One ml of culture was inoculated in the BAz broth and incubated for 7 days at room temperature. The cultures were centrifuged for 10 minutes at 10,000 rpm and the supernatant was taken. The cell pellet was re-extracted with phosphate buffer (pH 8.0) and again centrifuged. Both supernatants were collected and pooled, acidified to pH 2.0 with 5N hydrochloric acid and extracted with equal volumes of ethyl acetate twice [30]. The ethyl acetate phase was evaporated at 32°C and the residue was redissolved in 2 ml of distilled water containing 0.05 per cent of Tween 80.

2.4.2. Spectrophotometric Estimation of Gibberellins (GA_3)

Two ml of zinc acetate solution was added to the dissolved residue. After two min, two ml of potassium ferro cyanide solution was added and the mixture was centrifuged at 10,000 rpm for 10 min. Five ml of supernatant was added to 5 ml of 30 per cent hydrochloric acid and the mixture was incubated at 20°C for 75 min. The blank was prepared with 5 per cent hydrochloric acid. The absorbance was measured at 254 nm in spectrophotometer. From the standard gibberellic acid solution, the amount of GA produced by the culture was calculated and expressed as μg ml^{-1}.

2.5. Estimation of Cytokinin

2.5.1. Extraction from Culture Filtrates

The organisms were grown in BAz media until good growth and centrifuged at 10,000 rpm for 10 minutes. The cell free supernatants were collected and adjusted to pH 2.8 with 1N HCl. To the culture filtrate in separating funnel equal volume of cold (4°C) diethyl ether was added, mixed and allowed to stand for 4 hours at 4°C with intermittent shaking. The aqueous phase was separated from the organic phase and evaporated to dryness in the dark. The residue was dissolved in 2.0 ml of absolute alcohol and used for bioassay.

2.5.2. Radish Cotyledon Assay

Radish seeds were surface sterilized with 0.5% sodium hypochlorite solution and after rinsing with sterile water, it was allowed to germinate on blotter paper in darkness at 25-26°C for three days. Cotyledons of uniform weight were selected and placed on filter paper in 9 cm petriplates. Ten ml of 2 mM potassium phosphate buffer of pH 5.9 followed by one ml of cell free culture extract prepared earlier were added to petriplates. Similar set of experiments were conducted with various concentrations of Benzyl amino purine *viz*., 2, 4, 6, 8 and 10 μg ml^{-1} instead of culture extract. Cotyledons were incubated under continuous weak fluorescent light for three days at 24°C. The weights of the cotyledons were recorded after drying with blotting paper and a dosage response curve was drawn.

2.6. Determination of Siderophore Production

Siderophore production was estimated by the method described by [31]. BAz broth was prepared and dispensed in 100 ml quantities in 250 ml flasks and sterilized. One ml of *Burkholderia* isolates was added into above medium and incubated at 37°C for 7 days. After incubation, the broth culture was centrifuged at 10,000 rpm for 10 minutes. Twenty ml of culture supernatant was taken and the pH was adjusted to 3.0. To this 20 ml of ethyl acetate was added and extraction was done twice. The solvent was evaporated and then residue was dissolved using distilled water. It was used as assay solution for the estimation of catechol type and salicylic type of siderophore.

2.6.1. Salicylate Type of Siderophore

Five ml of the assay solution was added with 5 ml of Hathway reagent (1ml of 0.1 M $FeCl_3$ and 1 ml of 0.1 N HCl was added to 100 ml distilled water and to this 1 ml of 0.1 M potassium ferrocyanide was added) and absorbance was determined at 560 nm with sodium salicylate as standard for the estimation of salicylic type of siderophore.

2.6.2. Catechol Type of Siderophore

For the measurement of catechol type siderophore, 5 ml of Hathway reagent was added with 5 ml of assay solution and the absorbance was determined at 700 nm with 2,3, di hydroxy benzoic acid as standard.

2.7. Determination of ACC Deaminase (1-Amino-Cyclopropane-1-Carboxylic Acid) Activity

ACC deaminase activity of the cell was determined as per method described by [32]. One ml of the bacterial culture was added to 50 ml sterile PAF medium in a 250 ml flask. The flask and its contents were incubated in a shaking water bath (200 rpm) at a temperature between 25 and 30°C. After 24 hr, 1ml aliquot was removed from the growing culture, transferred to 50 ml of sterile PAF medium in a 250 ml conical flask and incubated at 200 rpm in a shaking water bath for 24 hr, at the same temperature as the first incubation (Following these incubation, the population of

Burkholderia and other bacteria were enriched and the number of fungi in the culture was reduced). One ml aliquot was removed from the second culture and transferred to 250 ml flask containing 50 ml sterile DF minimal salt medium in a 250 ml flask containing 3.0 mM ACC deaminase. The culture was placed in a shaking water bath at 200 rpm and grown for 24 hr at 25-30°C. The bacteria were harvested by centrifugation at 8000 g for 10 minutes at 4°C. The supernatant was removed and the cells were washed by suspending the cell pellet in 5 ml 0.1 M Tris-HCl, pH 7.6 and assayed for ACC deaminase activity by Spectrophotometer at 540 nm.

2.8. Statistical Analysis

All experiments were conducted in triplicates and mean and standard deviation were determined. Data sets were subjected to analysis of variance (ANOVA) on Windows Excel.

3. Results

3.1. Enumeration of Burkholderia sp. from Different Crops

The population of *Burkholderia* was enumerated from different parts of rice, maize, sugarcane and black gram and the data were presented in Table 1. The population was comparatively more in the root samples than stem and leaf of the crops studied. Black gram roots known to harbour more number of the endophyte (30 CFU g^{-1} tissue) followed by maize. The colonies of *Burkholderia* isolates were grown on BAz (*Burkholderia* Azeleic acid) medium were shown in Figure 1.

Table 1. *Population of Burkholderia sp. in different parts of crop plants.*

Crops	Population (CFU x 10^3 g^{-1} tissue)		
	Root	Stem	Leaf
Rice	12.5	8	1.5
Maize	25	9.5	2.5
Sugarcane	3.5	2.5	0
Black gram	30	19.5	6.5

Figure 1. *Growth of Burkholderia isolates on BAz agar medium.*

3.2. Production of IAA, GA and Cytokinin by Burkholderia Isolates

We were presenting detailed investigation of *Burkholderia* isolates of quantity of IAA produced without tryptophan varied between 1.80 and 3.61 µg ml^{-1}. Among the isolates, BB_4 produced higher quantity of IAA (3.61), which was followed by *B. vietnamiensis* (3.03), RB_1 (2.86), SB_3 (2.52) and MB_2 (2.35). Similarly, quantity of IAA produced with tryptophan varied from 2.77 to 5.06 µg ml^{-1}. Among the isolates, BB_4 produced higher quantity of IAA (5.06), followed by *B. vietnamiensis* (4.03), RB_1 (3.89), *Burkholderia tropicalis* (3.45), SB_3 (3.36) and MB_2 (2.07), the results were showed in Figure 2. Quantity of GA produced varied between 2.39 and 3.82 µg ml^{-1}. Among the isolates, BB_4 recorded higher quantity of GA (3.82), which was followed by *Burkholderia tropicalis* (3.69), RB_1 (3.11), MB_2 (2.76) and the lowest value was recorded in *B. vietnamiensis* (2.39), the results were shown in Figure 3. Cytokinin production was tested by measuring the weight increase of radish cotyledon due to culture extract of *Burkholderia* as compared to control. Among the isolates, *Burkholderia tropicalis* (0.463 g g^{-1}) recorded maximum increase in weight of radish cotyledon followed by SB_3 (0.388), BB_4 (0.357), *B.v* (0.327) and RB_1 (0.309) respectively. The lowest weight was recorded in MB_2 (Table 2).

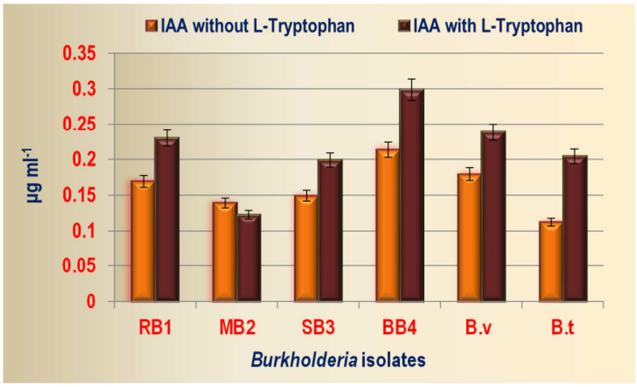

Figure 2. *Indole Acetic Acid (IAA) produced by Burkholderia isolates by using BAz medium.*

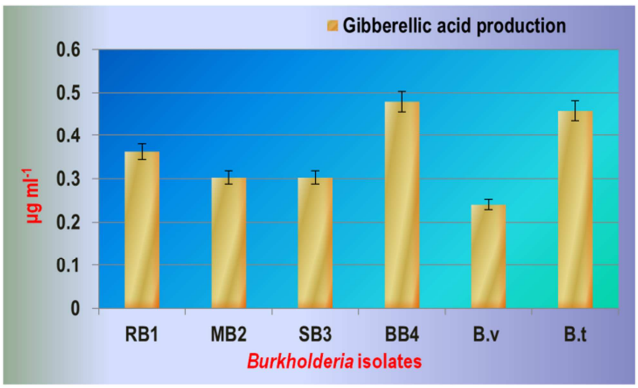

Figure 3. *Production of Gibberellic Acid (GA) by Burkholderia isolates grown on BAz medium.*

Table 2. *Production of Cytokinin by Burkholderia isolates grown on BAz medium.*

Burkholderia isolates	Cytokinin (mg g^{-1})		
	Weight of radish cotyledons before adding culture filtrate (g)	Weight of radish cotyledons after incubating in culture filtrate (g)	Average weight of radish cotyledons (g)
RB_1	0.102	0.411	0.309
MB_2	0.104	0.401	0.297
SB_3	0.104	0.492	0.388
BB_4	0.102	0.459	0.357
B. vietnamiensis*	0.098	0.425	0.327
B. tropicalis*	0.106	0.569	0.463
Control	0.108	0.299	0.191
SEd			0.014
CD (5%)			0.013

(* - Reference strains)

3.3. Production of Siderophore by Burkholderia Isolates

Siderophore production was recorded in all the isolates and in reference strains as well. The higher siderophore (Salicylate type) production was recorded in *Burkholderia tropicalis* (2.39 µ moles ml^{-1}), which was followed by *B. vietnamiensis* (2.30), RB_1 (2.23), BB_4 (2.22) and SB_3 (2.20). The lowest siderophore production was recorded in MB_2 (1.74). Similarly, in the case of catechol type siderophore production, the maximum value was recorded in BB_4 (1.72 µ moles ml^{-1}) followed by *Burkholderia tropicalis* (1.66), MB_2 (1.61), RB_1 (1.53), SB_3 (1.47) and the lowest value was recorded in *B. vietnamiensis* (1.45), both the results were presented in Figure 4.

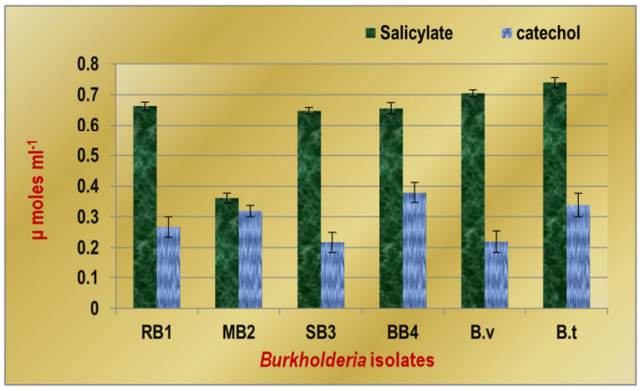

Figure 4. *Siderophore production of Burkholderia isolates.*

3.4. Production of ACC (1-Aminocyclopropane 1-Carboxylic Acid) Deaminase by Burkholderia Isolates

ACC deaminase activity was recorded in all the isolates and in reference strains. The highest value was recorded in *B. vietnamiensis* (1.32 nmoles h^{-1}) followed by BB_4, SB_3, *Burkholderia tropicalis* and MB_2. The lowest value was recorded in RB_1. The results were presented in Table 3.

Table 3. *ACC deaminase activity of Burkholderia isolates.*

Burkholderia isolates	ACC deaminase activity (n moles of α-Ketobutyrate mg^{-1} h^{-1})
RB_1	0.44
MB_2	0.58
SB_3	0.67
BB_4	1.18
*B. vietnamiensis**	1.32
*B. tropicalis**	0.64
SEd	0.03
CD (5%)	0.07

(* - Reference strains)

4. Discussion

The present study showed that the population of *Burkholderia* was comparatively more in the root samples than stem and leaf of the crops studied. Similar results were observed by [33] who reported more number of nitrogen fixing *Burkholderia* sp. in root tissues of maize than shoot. In another study *Burkholderia* represented 2-7 per cent of culturable bacteria isolated from soil and 17-25 per cent culturable bacteria from roots of sugarcane, indicating the close association between *Burkholderia* sp. and sugarcane root in the presence of other indigenous microorganisms [34]. Similarly more number of these bacteria was observed in the roots of rice as reported by [35]. Thus, it is clearly shown that *Burkholderia* are root endophytic organisms in various crop plants. As reports suggest their occurrence, various reports of effects of rhizophere organisms in crop improvement have been published.

Microorganisms in the rhizosphere of crop plants produce several phytohormones as plant growth regulating substances. Auxin is a key hormone for various aspects of plant growth and development, thus its synthesis by bacteria is probably one of the reasons for the alterations observed in plant metabolism. The presence of Indole acetic acid (IAA) in the methanolic extract from growth media of *B. brasilense* and *Herbaspirillum* were suggested by [36]. The present study showed that *Burkholderia* isolate BB_4 recorded higher production of IAA when compared to other isolates. Similar results were reported by [37] in *Stenotrophomonas maltophilia* for IAA production whereas *Pseudomonas putida* for GA (Gibberellic acid) and Cytokinin production. In present experiment, the isolates showed the IAA and GA production in significantly higher quantity. Similar to the present study reports suggesting microbial production of IAA and GA. Besides auxin production, *Pseudomonas fluorescence* was also found to produce gibberellic acid substance [38, 39]. It was observed that extremely high amount of IAA production by *Azospirillum* [40]. However,

the amount of IAA produced varies with the species and the condition of cultivation [41]. The strains of *Bacillus thuringiensis* C110*, Pseudomonas fluorescens* Th98 and *Pseudomonas poae* Th75 also proved to be effective PGPR inoculants [42]. *Pseudomonas Spp.* for K8 and *Azotobacter Spp.* for K37 isolates were produced higher amount of Gibberellic acid also identified with effect of pH, incubation temperature, carbon and nitrogen sources [43]. The endophytic *Burkholderia* isolates were produced by IAA and GA from cultivated sugarcane crops is used as growth regulator which may potent native for development of biofertilizer production [44].

Production of this type of plant growth regulators (IAA and cytokinin) have been earlier observed in *Rhizobium* [45], *Azotobacter* [46], *Azospirillum* [47, 28] and in phosphobacteria [48]. The *Burkholderia* isolates tested, produced varied quantities of IAA and cytokinins and it supported the findings of [49, 50]. They observed the auxin and cytokinin production by many root colonizing bacteria including *Pseudomonas* sp. The present study of raddish cotyledon bioassay revealed the production of cytokinin by *Burkholderia*. The amount of cytokinin produced varied among isolates. The reference strain *B. tropicalis* and the isolates BB_4 and SB_3 produced high amounts of cytokinin. The use of plant growth promoting rhizobacteria is an eco friendly approach to increase the crop growth to enhance the sustainable Agriculture [51].

Siderophore are relatively low molecular weight (< 1500 Kda) compounds. There are almost 500 compounds identified as siderophores. They can be divided into hydroxmate, catecholate, salicylate and hydroxy carboxylic acid type [52]. *Burkholderia cepacia* has been reported to produce four different types of siderophores *viz.,* pyochelin, salicylic acid (SA), cepabactin and ornibactins [53]. More recently, *B. cepacia* strains have also been reported to produce linear hydroxamate or hydroxycarboxylate siderophore termed as ornibactins [54]. Previously, catechol and salicylate type siderophores have been noticed in *Azospirillum* [55]. In the present experiment, *Burkholderia* isolates from different crops recorded 2.39 µ moles ml^{-1} of salicylate type of siderophore and 1.72 µ moles ml^{-1} of catechol type of siderophore under ion limiting condition, which is in accordance with the findings of [56]. The *B. cepacia* isolates which produce only ornibactins are more related to *B. vietnamiensis* and the production of siderophores may be used to discriminate between these two species [57] in a study suggested that clinical isolates of *B. cepacia* could be differentiated from rhizosphere isolates by their production of pyochelin and salicylic acid. Some of the newly isolated endophytic ACC deaminase strains were produced IAA, Siderophore production, phosphate solubilization activity, optimal growth temperature, salt tolerance and antibiotic sensitivity [58]. Thus, the isolate has been proven to be a versatile PGPR organism.

Moreover, a bacterium may utilize different traits at various times during the life cycle of the plant. PGPR may lower the plant ethylene concentration during seed germination thereby decreasing the ethylene inhibition of seedlings root length [59]. Once the seedling has depleted the resources that are contained within the seed, the same PGPR may help to provide the plant with iron and phosphorus from the soil. For many plants, a burst of ethylene is required to break seed dormancy [60]. But, following germination, a sustained high level of ethylene would inhibit root elongation. PGPR that contain the enzyme ACC deaminase, when bound to the seed coat of a developing seedling act as a mechanism for ensuring that the ethylene level does not become elevated to the point where initial root growth is impaired. Growth of N_2 fixing *Burkholderia* isolates using 1-aminocyclopropne-1-carboxylic acid (ACC) as the sole nitrogen source was tested on BAz-ACC medium [22]. In the present study, the ACC enzyme of 1.32 n moles h^{-1} was produced by *B. vietnamiensis* and followed by local isolate BB_4 (1.18 n moles h^{-1}). ACC deaminase has an ability to facilitate plant growth to a much greater extent with plants that are ethylene sensitive such as canola, pepper and tomato, and their activity is useful in Agricultural and Horticultural settings as well as other environmental cleanup [32]. Acc deaminase activity was noticed in *Bacillus subtilis* as bacterial endophytes [61].

5. Conclusion

The present study depicted that the significant PGPR activities of *Burkholderia* isolates has clearly proven from the results *viz*., Siderophore production, significant production of growth promoting substances and ACC deaminase activity. Hence the study, putforth *Burkholderia* isolates as a potential PGPR organism for crop plants and they can be efficiently utilized for crop production. However, further studies on the isolate regarding method of application will help in reaching the technology to field level.

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