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Role of *Bacillus aryabhattai* in plant growth and development

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ABSTRACT

Plants produce a wide range of organic compounds like sugars, organic acids and vitamins, which can be used as nutrients or signals by microbial populations. On the other hand, microorganisms release phytohormones, enzymes, which may act directly or indirectly to activate plant immunity and regulate plant growth. Plant signalling molecules such as auxin and cytokinin can be produced by microorganisms to colonize efficiently with roots and enhance root activity. The isolated microbe *Bacillus aryabhattai*, has the ability to produce1-aminocyclopropane-1-carboxylate (ACC) deaminase to lower plant ethylene level, often a result of various stresses, and is found to be a key component in the efficacious functioning of this bacterium. The optimal functioning of this bacterium includes the synergistic interaction between ACC deaminase, and plant with bacterial indole-3-acetic acid (IAA).

Key words: ACC deaminase, *Bacillus aryabhattai*, Induced Systemic Tolerance.

INTRODUCTION

Extensive communication occurs between plants and microorganisms during different stages of plant development in which signalling molecules from the two partners play an important role (Randy, 2009). Fungal and bacterial species are able to detect the plant host and initiate their colonization strategies in the rhizosphere by producing canonical plant growth-regulating substances such as auxin, cytokinin and enzymes. On the other hand, plants are able to recognize microbe-derived compounds and adjust their defence and growth responses according to the type of microorganism encountered (Randy, 2009). Beneficial soil bacteria and fungi can confer immunity against a wide range of foliar diseases by activating plant defences, thereby reducing a plant's susceptibility to pathogen attack (Randy, 2009). For many years, this was considered the basis by which beneficial microorganisms could increase plant yield when inoculated in crops. However, it is increasingly appreciated that classic and novel microbial signals may also directly participate on plant morphogenesis.

The bacterial trait that is key in facilitating plant growth is the possession of the enzyme ACC deaminase. This enzyme is responsible for the cleavage of the plant ethylene precursor, ACC into ammonia and α-ketobutyrate (Francisco, 2014). By decreasing ACC levels in plants, ACC deaminase-producing organisms decrease plant ethylene levels, which when present in high concentrations can lead to plant growth inhibition or even death. ACC deaminase-producing plant growth-promoting bacteria first bind to the surface of a plant (usually seeds or roots), although these bacteria may also be found on leaves and flowers or within a plant's internal tissues as an endophyte. Plants typically exude a large fraction of their photo synthetically fixed carbon (estimated to generally be in the range of 5–30%) through their roots. Root exudates generally contain large amounts of sugars, organic acids and amino acids, and the ability of these compounds to act as a bacterial food source is the main reason why the numbers of bacteria around the rhizosphere are 10–1000 times higher than in the bulk soil. In response to the presence of tryptophan and other small molecules in the plant root exudates, the associated bacteria synthesize and secrete the phytohormone indole-3-acetic acid (IAA), some of which is taken up by the plant. This IAA, together with endogenous plant-synthesized IAA can affect plants in several different ways. It can stimulate plant cell proliferation or plant cell elongation, or it can induce the transcription of the plant enzyme ACC synthase that catalyzes the formation of ACC (Francisco, 2014). In this case, IAA acts to stimulate the synthesis of ethylene in the plant.

The net result of the cleavage of exuded ACC by bacterial ACC deaminase is that the bacterium is de facto acting as a sink for ACC (Glick, 2014). ACC deaminase-containing plant growth-promoting bacteria (PGPR) can reduce a portion of the ethylene inhibition of plant growth following a wide range of abiotic and biotic stresses. As a result, plants which grow in association with ACC deaminase producing PGPR generally have longer roots and shoots and are more resistant to growth inhibition by a variety of ethylene-inducing stresses (Dinesh, 2015). Stress induces the induction of ACC oxidase in the plant so that there is an increased flux through ACC oxidase resulting in the first peak of ethylene that in turn induces the transcription of protective/defensive genes in the plant (Bernard, 2014) (Fig 3). At the same time, bacterial ACC deaminase is

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induced by the increasing amounts of ACC that ensure from the induction of ACC synthase in the plant so that the magnitude of the second, deleterious, ethylene peak is decreased significantly (typically by 50–90%) (Dinesh, 2011). Because ACC oxidase has a greater affinity for ACC than does ACC deaminase, when ACC deaminase-producing bacteria are present, plant ethylene levels are dependent upon the ratio of ACC oxidase to ACC deaminase. That is, to effectively reduce plant ethylene levels, ACC deaminase must function before any significant amount of ACC oxidase is induced. A naive view of the interaction of IAA-producing bacteria with plants might posit that since IAA activates the transcription of ACC synthase, these bacteria should all ultimately result in the production of relatively high concentrations of ACC and subsequently inhibitory levels of ethylene (Glick 2014).

MATERIALS AND METHODS

The soil samples were collected from different places. The well grown plant with intact roots was uprooted from the field and excess soil was removed. The soil adhered to root surface and in between root was collected and used as rhizosphere soil for the PGPR isolation. 1g of soil was dissolved in 10ml distilled water, heat treated at 80ºC for 15-20 minutes (Travers, 1987). The soil suspension was serially diluted and appropriate dilutions were spread plated on L-acetate agar (Assaeedi, 2011). The plates were incubated at 30±2°C and isolated colonies were selected for further studies. Eighteen isolates were successfully isolated and individually sub-cultured. Among which 8 were selected based on IAA production ability. The isolates were coded as S1, S2, S3, S4, S5, S6, S7, and S8.

IAA production by each isolates was determined by colorimetric technique, performed with Van Urk Salkowski reagent using the Salkowski's method (Mohite, 2013). The isolates were grown in yeast malt dextrose broth (YMD broth) and LB media (Himedia, India) and incubated at 30°C for 7 days. The broth was transferred to centrifuge tube and centrifuged @ 10000 rpm for 10min. Supernatant was collected and to 1ml was mixed with 2ml of Salkowski's reagent (2% 0.5 $FeCl_3$ in 35% $HCLO_4$ solution) (Szkop, 2011) and kept in the dark. The colour change to pink/red indicated the presence of IAA. The optical density (OD) was recorded at 530 nm after 30 min and 120 min. IAA production was compared in YMD and LB media. YMD medium was compared with and without tryptophan. Among these 8 isolates, S6 gave highest intensity of colour. Estimated quantity IAA produced was 150µg/ml. (Fig 1).

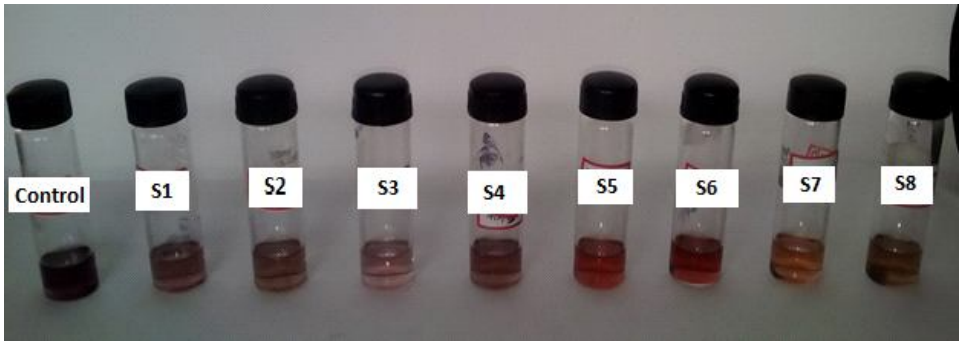
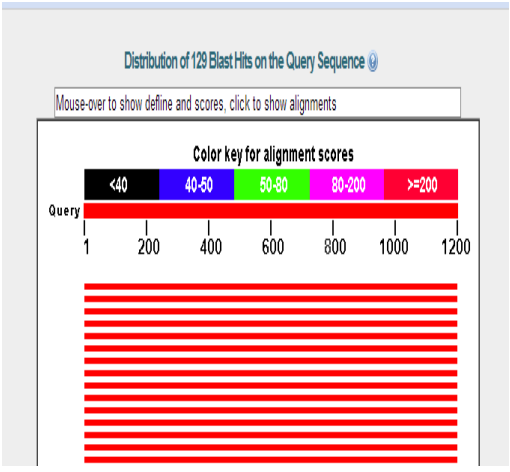


Fig 1: Different isolates and their IAA production.



>*B. aryabhattai*

GGTTGCTGGCGGCGTGCTTAATACATG
CAAGTCGAGCGAACTGATTAAAGCTT
GCTTCTATGACGTTAGCGGCGGACGGG
TGAGTAACACGTGGGCAACCTGCCTG
TAAGACTGGGATAACTTCGGTAAACCG
AAGCTAATACCGGATAGGATCTTCTCC
TTCATGGGAGATGATTGAAAGATGGTT
TCGGCTATCACTTACAGATGGGCCCGC
GGTGCATTAGCTAGTTGGTGAGGTAAC
GGCTCACCAAGGCAACGATGCATATCC
GACCTGAGAGGGTGCCCGGCCCTTCA
GGGACTGAGACACGGCCCACACTCCT
AAGGGAGGCAGCAGTAGGGAATCTTC
CGCAATGGACGAAAGTCTGACGGAGC
AACGCCGCGTGAGTGATGAAGGCTTT

Fig 2: BLAST alignment and Neucleotide Sequence of B.aryabhattai.

Screening for ACC deaminase: All the isolates, were grown in 5 ml of TSB medium incubated at 28ºC at120 rpm for 24 h. The cells were harvested by centrifugation at 3000 rpm for 5 min and washed twice with sterile 0.1 M Tris-HCl (pH 7.5) and resuspended in 1 ml of 0.1 M Tris-HCl (pH 7.5) and spot inoculated on petri plates containing modified DF salts minimal medium (Foster, 1958), glucose, 2.0 g; gluconic acid, 2.0 g; citric acid, 2.0 g; KH_2PO_4, 4.0 g; Na_2HPO_4, 6.0 g; $MgSO_4$.7H2O, 0.2 g; micro nutrient solution ($CaCl_2$, 200 mg; $FeSO_4$.7H2O, 200 mg; H_3BO_3, 15 mg; $ZnSO_4.7H_2O$, 20 mg; Na_2MoO_4, 10 mg; KI, 10 mg; NaBr, 10 mg; $MnCl_2$, 10 mg; $COCl_2$, 5 mg; $CuCl_2$, 5 mg; $AlCl_3$, 2 mg; $NiSO_4$, 2 mg; distilled water, 1000 ml), 10 ml and distilled water, 990 ml; supplemented with 3 mM ACC as sole nitrogen source. Plates containing only DF salts minimal medium without ACC as negative control and with $(NH4)_2SO_4$ (0.2% w/v) as positive control. The plates were incubated at 28ºC for 72 h. Growth of isolates on ACC supplemented plates was compared to negative and positive controls and was selected based on growth by utilizing ACC as nitrogen source.

DNA isolation: DNA isolation was done by using modified CTAB protocol according to (Scheie, 2000), modified. Take 2ml culture broth and centrifuged at 13000 g for 10 min, and the supernatant was discarded. To the pellet, add 500µLof extraction buffer. Add proteinase K and lysozyme 50µL each for 2ml sample. Mix gently and keep 30 min water bath at 37ºC. Add 5µL potassium acetate and 50µL SDS and mix gently. Keep at water bath 30min at 65ºC by mixing every 10 min. Cool for 5min at room temperature. Add 500 µL of chloroform:IAA (24:1ratio). Vortex it for few second. Then centrifuge at 13000 g for 15 min. Take 400µL of supernatant and add chloroform:IAA(1:1). Centrifuge at 13000 g for 10 min. Take 200µLof supernatant and add IPA equal volume and mix gently. Centrifuge at 13000 g for 10 min. Discard the supernatant and add 200µLof ethanol (70%). Tap the tube and centrifuge at 13000 g for 5 min. Discard the supernatant and keep for dry bath at 65ºC. Add TE+RNAse 20µL and kept 15 min at dry bath. Then DNA was ribotyped and found to be *Bacillus aryabhattai*, (Fig 2).

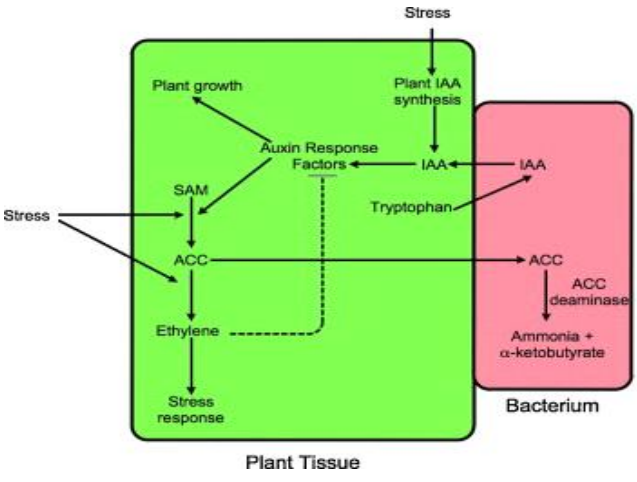


Fig 3: Interlinked mechanism between plant root exudate and ACC deaminase producing microbes. Bernard R. Glick. (2014).

Pot Assay: To study the effect of IAA and ACC deaminase producing rhizospheric isolates on plant growth, pot assay was performed. Local chili seeds were used for seed coating. The chili seeds were surface sterilized by immersing in 95% ethanol for 30 sec and mercury chloride (0.2%) for 3 min. Then further to remove traces of mercury chloride, the disinfected seeds were washed 5 times by sterile distilled water (Mohite, 2013). 0.1ml 7 days grown culture was applied on seed surface for seed coating. Seeds were dried and sowed into sterile soil as carrier in each pot and experiment was performed in triplicates for S6 isolate. The uncoated seeds were used as control.

After seedlings, pots were irrigated with sterile distilled water every day and kept in sunlight. At the interval of every 5th day, plant was treated with different dosage of 2ml, 3ml and 5ml. After 30 days, plants were uprooted and plants were measured for number of branches, leaf wideness, stem grid and root length. (Table 1 and Fig 4 and 5).

RESULTS AND DISCUSSION

The isolates were screened for ACC deaminase based on the enrichment method, where ACC was used as the sole nitrogen source (Shaik Zulfikar, 2013). Among all the isolates, S6 grew well on DF salt minimal medium with either ACC or ammonium sulfate serving as the sole nitrogen source which was compared with DF salt minimal medium without nitrogen source. Isolate which was positive for ACC deaminase was shortlisted and subjected to screening for plant growth promoting traits. The isolate also produced significant amount of indole acetic acid (IAA) and exhibited phosphate solubilisation.

The shortlisted isolate was ribotyped and found to be *Bacillus Aryabhattai*. Our aim is rather to discuss recent findings about the signals involved in the interaction of

Table 1: In-house pot trial observation chilli plant with different dosage of S6 broth.

Observation	Number of Branches	Number of Leaves	Stem Thickness	Root Length	Plant Weight
Control	0	12	1.50cm	5.0cm	3.52g
2ml(Test)	6	28	3.20cm	10.0cm	10.12g
3ml(Test)	6	26	3.01cm	9.0cm	9.05g
5ml(Test)	6	25	2.75cm	8.5cm	8.20g

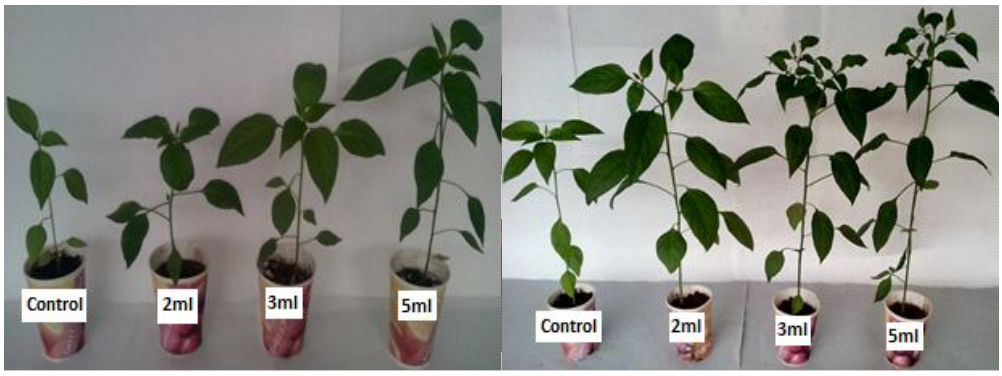


Fig 4: Growth comparison test with different dosages by drenching.



Fig 5: Growth comparison between control and test plants.

plants with free-living, beneficial microbes (PGPR), for which important recent discoveries have been made on their chemical communication, the biological processes they sustain and the benefits to plants involved in these interactions.

CONCLUSION

Plants and microorganisms have coexisted for millions of years. Plants maintain a complex interaction with their rhizospheric populations, which is crucial for nutrient assimilation, development and activation of defence mechanisms. These mutually beneficial associations are possible because plants and microorganisms can communicate with each other through various signaling mechanisms. To feed all of the world's people, it is necessary to sustainably increase agricultural productivity. One way to do this is through the increased use of plant growth-promoting bacteria; recently, scientists have developed a more profound understanding of the mechanisms employed by these bacteria to facilitate plant growth. Here, it is argued that the ability of plant growth-promoting bacteria that produce 1-aminocyclopropane-1-carboxylate (ACC) deaminase to lower plant ethylene levels, often a result of various stresses, is a key component in the efficacious functioning of these bacteria. The optimal functioning of these bacteria includes the synergistic interaction between ACC deaminase and both plant and bacterial IAA. These bacteria not only directly promote plant growth, but they also protect plants against flood, drought, salt, flower wilting, metals, organic contaminants, and both bacterial and fungal pathogens. The evidence indicates that with the expected shift from chemicals to soil bacteria, the world is on the verge of a major paradigm shift in plant agriculture.

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