Evaluation of ACC Deaminase producing bacteria over non-producers in protecting *Vigna radiata* growth under Salt and Boron stress

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Abstract
The 1-aminocyclopropane-1-carboxylate deaminase (ACCD) is a crucial bacterial trait found in rhizobacteria for amelioration of different plant stresses. A co-inoculation approach is explored in this study. Our objective was to compare the efficacy of PGPB with ACCD activity and without ACCD activity in improving the growth of legume Vigna radiata under salt and boron stress. Two isolates were selected for comparative study in the mitigation of salt and boron stress with and without ACCD activity. Seeds of Vigna radiata were co-inoculated with TKP-A3 having ACCD activity and BVB-4 lacking ACCD exposed with two different concentrations of boron (6.5mM and 7.5mM H_3BO_3) under saline stress (40 mM NaCl). Our findings confirm that the detrimental effects due to excess salt and boron were successfully mitigated in plants co-inoculated with the TKP-A3 as compared to BVB-4. Our results have shown that salt and boron stress significantly altered the lateral root formation, root length, chlorophyll content and vigor index of the seedlings.

Interestingly, the addition of TKP-A3 significantly increased shoot length, vigor index and chlorophyll content by almost 15%, 16% and 50% respectively compared to BVB-4, indicating the importance of ACCD activity in the mitigation of salt and boron stress. Additionally, no positive effects were observed on different growth parameters when seeds were coated with BVB-4 alone. This study highlights the significance of ACCD producing PGPB over PGPB lacking ACCD to improve growth characteristics of Vigna radiata under salt and boron stress. Molecular analysis identified TKP-A3 and BVB-4 as Enterobacter quasiroggenkampii and Cytobacillus pseudoceanisediminis respectively.

Keywords: ACC deaminase, PGPB, Saline stress, Boron stress**,** *Cytobacillus pseudoceanisediminis, Enterobacter quasiroggenkampii.*

Introduction
Since the start of the twenty-first century, environmental pollution, shortage of water resources and rising soil and water salinity have received increased attention worldwide. Salinized soil is one of the most important and detrimental environmental elements that reduce crop quality and production in cultivated regions[28]. In addition to being a crucial element in plant development and production, excess salinity also causes a lowering of agricultural output and yield. Soils impacted by salt have been a major crop-limiting problem worldwide[39]. The growing human population and the declining amount of land suitable for farming pose threats to sustainable agriculture[7]. The main factors contributing to losses in over-irrigated areas with extreme use of synthetic fertilizers are drought and excessively salinized soil and the salinity-affected land area is expanding every day[11].

Both natural and man-made sources discharge boron (B) into terrestrial and aquatic habitats. Waste from the mining and processing industries, B fertilizer application and wastewater for irrigation are examples of anthropogenic sources of B released into the environment[35]. For plants to survive, boron is a necessary element that is mostly absorbed as the molecule boric acid. Though there has been evidence of B deficit in agricultural soils, B toxicity can prevent plant growth in soils found in arid and semi-arid areas[4]. In order to meet the food demands of the anticipated year 2050 population, yields of key crops will need to increase significantly (estimated at a rate of 50%)[28]. Developing the right agricultural techniques is crucial and needs to be done to stop these issues. The development of short-term, simple biological approaches for regulating salt and boron stress is required due to some lengthy, costly and uncertain processes.

In these conditions, research needs to apply appropriate biotechnological techniques to increase both crop yields and enhance soil health via the interaction of plant roots and soil bacteria[26]. It is widely identified that soil stress caused by salt inhibits plant growth. However, boron stress has not yet been reported, yet it poses significant toxicity in plants experimentally. It is commonly recognized that plants exposed to such stresses benefit from the presence of soil biota. Plant growth-promoting (PGP) rhizobacteria (PGPR) have been employed to accomplish this goal by isolating microorganisms found in the rhizosphere of saltwater plants[18]. According to earlier research, using PGPRs is a viable substitute for treating plant stress brought on by salinity[43]. As a result, microbes are playing an increasingly significant role in managing biotic and abiotic stresses.

To reduce yield losses from salt stress, this work will be useful in examining the potential of native salt-tolerant strains of PGPR and advancing their use as biofertilizers for wheat crops. In the future, for such problematic soil, development of an effective bioformulation will be needed[9]. The ACCD enzyme received special consideration due to its significance in the nodulation process and in regulating the internal ethylene levels in legume roots. Gaseous phytohormone ethylene plays a variety of roles in the nurturing of healthy plants in a certain amount[8]. Ethylene is produced in modest amounts in certain conditions. Due to environmental pressures its production in plant organs is induced to increase dramatically. This can give rise to a variety of negative effects including reduced chlorophyll content, suppression of root elongation and nodulation and occasionally even plant mortality[40].

By converting ethylene precursor (ACC) into α-ketobutyrate and ammonia, which soil microorganisms can use as a carbon and nitrogen source, rhizobia that produces ACCD can lessen the harmful effects of ethylene stress on legume tissues[22] and promotes legume growth and symbiosis under stress conditions. Thus, stressful circumstances encourage legume development and symbiosis[40]. In this situation, halotolerant PGPRs could be very important in increasing wheat crop productivity unless their inherent traits such as salt tolerance, genetic diversity, production of osmoprotectant solutes, production of plant growth-promoting hormones, potential for biocontrol and interactions with cultivated plants are fully utilized[29].

The current study investigates the effects of two selected PGPB, having and lacking ACCD activity to promote and improve plant growth under salt and boron stress. We tested the ability of TKP-A3 having ACC deaminase to overcome salt and boron stress, in comparison with BVB-4 lacking ACC deaminase on *Vigna radiate.*

Material and Methods

Sampling: PGPB were isolated from the salt-affected soils of two agroclimatic zones of Maharashtra, India. Isolation was obtained by the dilution plating technique on soil extract agar plate (Hi-Media), purified and checked for their cultural and morphological characteristics by routine microbiological methods.

Isolation of IAA-producing and ACC-utilizing bacteria: The indol-3 acetic acid (IAA) production was quantified by Salkowsky's method at 530 nm by photometry[23]. Isolates showing IAA production were checked for ACCD activity by the method adopted by Penrose and Glick[33]. ACC utilization was verified by observing bacterial growth in M-9 mineral salt (M9-MS) media with 3 mM ACC (TCL Japan) as only a nitrogen and a carbon source.

Molecular identification and phylogenetic analysis: The isolates DNA was extracted as per the protocol provided by the manufacturers of the DNA isolation kit of Qiagen, USA. The 1.5 Kb of 16S rRNA gene amplification was performed with Universal forward and reverse primers as follows: 16F27 [-CCA GAG TTT GAT CMT GGC TCA G-3'] and 16R1492 [5'-TAC GGY TAC CTT GTT ACG ACT T-3'] with standard procedure. The amplified 16S rRNA gene PCR product was purified by PEG-NaCl precipitation and directly sequenced on an ABI® 3730XL automated DNA sequencer (Applied Biosystems, Inc., Foster City, CA) as per manufacturer's instructions. Essentially, sequencing was carried out from both ends using additional internal primers so that each position was read at least twice. Assembly was carried out using the Lasergene package followed by identification using the NCBI database[49].

PGP traits of two selected isolates: The phosphate solubilization by isolates was screened on Pikovskaya agar plates amended with inorganic phosphate[20]. Siderophore production was screened on chrome azurol S agar (CAS) medium[36]. A clearance zone was observed around the colonies, characterized by a transparent zone for phosphate solubilization and an orange zone for siderophore production, indicated a positive result for both PGP traits. The phosphate solubilization index (PSI) was determined using the formula (zone diameter + colony diameter)/colony diameter)[22]. Screening of atmospheric nitrogen fixation was evaluated by inoculating the isolates on Ashbys and Norris N_2 free medium at 28˚C for 2 days[21]. Visible growth in the form of colonies on respective media indicated N_2 fixation activity by the isolates. Potash solubilization ability was screened by using Aleksandrow medium (Hi-media) plates.

Culture suspensions were spot inoculated on Aleksandrow agar plates incubated for 24-48 hours at 28ºC. After incubation, growth as colonies with clear zone around indicated potash solubilization[32]. Zinc solubilization activity was detected by using M9-MS medium plates containing zinc oxide[3]. Culture suspensions were spot inoculated on zinc oxide medium plates incubated for 24-48 hours at 28º C. After incubation, a clear zone around the colony indicated potash and zinc solubilization. The antifungal activity of isolates was determined by screening chitinase production[47]. Medium containing colloidal chitin was spot inoculated with culture suspensions and incubated at 28ºC for 24 hours to observe a hydrolysis zone around the colonies indicating chitinase production using Gram's iodine.

Salt-tolerance determination of isolates: Pure cultures of isolates were inoculated in nutrient broth (5.0 mL/ tube) supplemented with final concentrations of NaCl as 5, 6,7,8,9 and 10 % (w/v) and incubated on rotary shaker (180 rpm) at 30°C. Bacterial growth was observed by checking the presence of turbidity in comparison with the uninoculated control.

Boron tolerance determination of isolates: Boron tolerance of both isolates was evaluated by observing bacterial growth in nutrient broth amended with different H_3BO_3 concentrations. Bacterial suspensions were inoculated in 5.0 ml of nutrient broth with H_3BO_3 concentrations, 25, 50, 75, 100, 200 and 300 mM. and incubated for 24 hours at 28°C. For each set of tubes, an uninoculated negative control was kept.

Temperature tolerance determination of isolates: The heat tolerance of both isolates was checked by observing the growth of bacteria incubated under different temperature conditions. Nutrient broth was used to grow the isolates and incubation temperatures were 35, 40, 42.5 and 45°C. A negative control for each set of temperatures was maintained.

Seed germination trials with selected isolates: Selected *Vigna radiata* (Mung Sindhu NVL 605) seeds were surface sterilized by using 1% NaOCl, then 30 seconds in 70% ethanol, rinsing twice in sterile distilled water and air drying in a laminar airflow environment. Log phase culture of TKP-A3 and BVB-4 with an OD_{580} 0.06 was used to coat the seeds. Treated seeds were sown on a 0.8% agar bed prepared with Hoagland nutrient solution. Salt and boron stress was given by incorporating the agar bed with final concentrations of 40 mM NaCl with 6.5 and 7.5 mM H_3BO_3. Seeds were germinated under dark conditions for 3 days. Three days old seedlings were subjected to a photoperiod of 10-12 hours for the day cycle and 10-12 hours for the night cycle for next 7 days. Each treatment had 45 seeds per plate in two replications.

After 7 days, the seedlings were harvested, washed carefully in tap water to remove any agar trapped in the roots and subjected to measurements of growth parameters like lateral root number, root length, shoot length, vigor index calculations etc. Chlorophyll (Chl) content of seedlings was quantified as follows: 0.1 g portion of fresh leaves was added in 1 ml of 80% acetone in duplicate, kept overnight at 4°C for extraction and centrifuged at 8000 g for 5 minutes and the absorbance was observed using a UV-spectrophotometer at 645 and 663 nm. A, Chl. *a*, Chl. *b* and total chlorophyll contents were calculated according to the formulae given in the procedure[34].

Statistical analysis: Statistical analyses were performed using Minitab software version 17. Data from the plant trial was analyzed using One-way Analysis of Variance (ANOVA) and the post-hoc test Tukey was used to compare the significant differences ($p < 0.05$) of bacterial treatments in terms of features of plant growth under salt and boron stress.

Results

Isolation of ACC utilizing bacteria and enzymatic assay: A total of 21 isolates were obtained from the collected soil samples of two agro-climatic zones. All isolates were primarily screened for IAA production out of which 10 isolates showed positive results (data not shown) that were further subjected to detect ACCD activity. During the screening for ACC utilization, a total of ten different types of morphologies were isolated and screened. However, only one isolate BVB-4 was unable to show ACCD activity (Table 1).

Molecular identification and phylogenetic analysis: The NCBI database's BLAST analysis showed that the 16S ribosomal RNA genomic sequence of the TKP-A3, BVB-4 showed 99.36 and 98.14 % similarity with *Enterobacter quasiroggenkampii* (Acc. No. PQ459551) and *Cytobacillus pseudoceanisediminis* (Acc. No. PQ456034) respectively.

PGP traits of two selected isolates: Table 1 shows all the qualitative and quantitative results of PGP screening. Both the isolates were able to solubilize phosphate and potash with an almost equal solubilization index, but zinc was only solubilized by *Enterobacter quasiroggenkampii*. Nitrogen fixation activity was shown by *E. quasiroggenkampii* on both Norris and Ashbys media, while *C. pseudoceanisediminis* fixed nitrogen on only Norris medium. *E. quasiroggenkampii* and *C. pseudoceanisediminis* were able to produce siderophores and chitinase showing their potential as biocontrol agent where *E. quasiroggenkampii* showed increased chitin hydrolysis index compared to *C. pseudoceanisediminis*.

Table 1
Screening of selected isolates for plant growth-promoting traits

Isolate	IAA production (ug/ml)	ACCD production	Phosphate solubilization	N_2 Fixation (Norris Medium)	N_2 Fixation (Ashbys Medium)	Potash solubilization	Zinc solubilization	Siderophore production	Chitinase
E. quasiroggenkampii	9.0	+	+ **(1.87)***	+	+	+ **(2.0)***	+ (4.0)*	+	+ (3.7)*
C. pseudoceanisediminis	16.0	-	+ **(1.75)***	+	-	+ **(2.0)***	-	+	+ (2.4)*

*value in bracket indicates solubilization index, Positive (+), Negative (-)

Salt-tolerance determination of isolates: Both *E. quasiroggenkampii* and *C. pseudoceanisediminis* could tolerate 8 % NaCl concentration showing a moderate halophilic nature (Table 2).

Boron-tolerance determination of isolates: Though 300 mM H_3BO_3 concentration was used as the highest one for boron tolerance, *E. quasiroggenkampii* and *C. pseudoceanisediminis* could grow only up to 75 mM concentration which is still much more toxic to all the types of plants (Table 3).

Temperature-tolerance determination of isolates: *C. pseudoceanisediminis* could grow at 42.5^{0}C and *E. quasiroggenkampii* at 40^{0}C confirming their mild thermophilic and mesophilic nature respectively (Table 4).

Seed germination trials with selected isolates: Two trials on model plant were conducted under different stressed conditions of salt and boron to study the impact of ACCD producer and non-producer bacterial isolate in co-inoculation with *Vigna radiata* seeds. In four treatments, the first one control seeds were uncoated with selected isolates and used to compare the treated ones. The second and third treatments were with *E. quasiroggenkampii* and *C. pseudoceanisediminis* coated seeds respectively. The fourth treatment contains a combination of *E. quasiroggenkampii* and *C. pseudoceanisediminis* both coated seeds. After 7 days, seedlings from all treatments were harvested, cleaned and tested for different growth parameters.

The plants inoculated with the *E. quasiroggenkampii* as being ACCD producer exhibited a substantial increase in shoot length and vigour index solely and in consortium with *C. pseudoceanisediminis* under both stress conditions. These findings emphasize the beneficial effects of ACCD in *Vigna radiata* growing under salt and boron stress (Tables 5 and 6). Interestingly, none of the treatments of the isolates had significant beneficial effects on lateral root formation and root length of *Vigna radiata*. Seeds co-inoculated with *E. quasiroggenkampii* and *C. pseudoceanisediminis* displayed increased chlorophyll content compared to control under both stressed conditions. Markedly, increased shoot length was observed in plants co-inoculated with *E. quasiroggenkampii* and its consortium with *C. pseudoceanisediminis* showing statistically significant mean values than those inoculated with *C. pseudoceanisediminis* alone and Control.

Moreover, the Vigor index of these plants surpassed that of plants inoculated with *C. pseudoceanisediminis* alone or uninoculated control. This significant enhancement in the vigour index suggests that the synergistic interaction between *Vigna radiata*, *E. quasiroggenkampii and* its consortium with *C. pseudoceanisediminis* not only enhances seedlings' tolerance to salt and boron stress due to the presence of ACCD but also improves the quality of growth. A comparative observation of seedlings under normal and stressed conditions is shown in figures 1 and 2.

Discussion

Excessive use of irrigation water results in the accumulation of salt and is also accompanied by boron in agricultural land which reduces crop yield and eventually results in barren land[10].

Table 2
Screening of selected isolates for salt tolerance

S.N.	Treatment	NaCl concentration (%)					
	Isolate	**5**	**6**	**7**	**8**	**9**	**10**
1	*E. quasiroggenkampii*	+	+	+	+	-	-
2	*C. pseudoceanisediminis*	+	+	+	+	-	-

Growth (+), No Growth (-)

Table 3
Screening of selected isolates for Boron tolerance

S.N.	Treatment	H_3BO_3 concentration (mM)					
	Isolate	**25**	**50**	**75**	**100**	**200**	**300**
1	*E. quasiroggenkampii*	+	+	+	-	-	-
2	*C. pseudoceanisediminis*	+	+	+	-	-	-

Growth (+), No Growth (-)

Table 4
Screening of selected isolates for Thermotolerance

S.N.	Treatment	Temperature (º Celsius)			
	Isolate	**35**	**40**	**42.5**	**45**
1	*E. quasiroggenkampii*	+	+	-	-
2	*C. pseudoceanisediminis*	+	+	+	-

Growth (+), No Growth (-)

Table 5
Seed germination trials of Green gram (Sindhu) NVL605 variety with TKP-A3 and BVB-4 under stressed conditions of salt and boron (40mM NaCl + 6.5 mM H_3BO_3).

Parameters	**Average Root no**	**Average Root length (cm)**	**Average Shoot length (cm)**	**Vigour Index**	**Percent germination (%)**	**Total Chlorophyll (mg/ml)**
Control Stress (40mM NaCl + 6.5 mM H_3BO_3)	**0^{a}**	3.03^{a}	3.52^{bc}	584.2^{b}	100	7.05^{b}
E. quasiroggenkampii	**0^{a}**	2.81^{a}	**4.06^{a}**	**679.1^{a}**	97.77	10.84^{a}
C. pseudoceanisediminis	**0^{a}**	2.14^{b}	3.24^{c}	524.5^{c}	94	7.18^{b}
E. quasiroggenkampii + *C. pseudoceanisediminis*	0^{a}	2.95^{a}	3.65^{bc}	**654.1^{a}**	98.88	6.28^{b}

Values in the parentheses are standard errors and parameters were recorded at 8 DAS.
Values superscribed by the same alphabet are not significantly different according to Tukey test ($P< 0.05$).

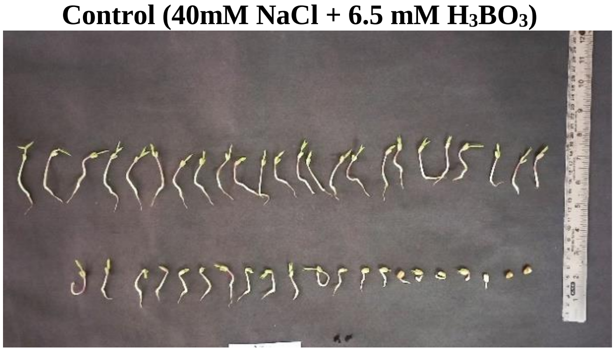
Table 6
Seed germination trials of Green gram (Sindhu) NVL605 variety with TKP-A3 and BVB-4 under stressed conditions of salt and boron (40mM NaCl + 7.5 mM H_3BO_3).

Parameters	**Average Root no**	**Average Root length (cm)**	**Average Shoot length (cm)**	**Vigour Index**	**percent germination (%)**	**Total Chlorophyll (mg/ml)**
Control Stress (40mM NaCl + 7.5 mM H_3BO_3)	**0^{a}**	**2.60^{a}**	3.35^{b}	597.9^{b}	**100**	5.06^{b}
E. quasiroggenkampii	**0^{a}**	2.51^{a}	**3.84^{a}**	**610.0^{ab}**	**95.5**	9.47^{a}
C. pseudoceanisediminis	**0^{a}**	2.02^{b}	3.182^{b}	564.9^{b}	**94**	8.9^{b}
E. quasiroggenkampii + *C. pseudoceanisediminis*	0^{a}	2.51^{a}	**3.81^{a}**	**621.1^{a}**	95.85	6.97^{b}

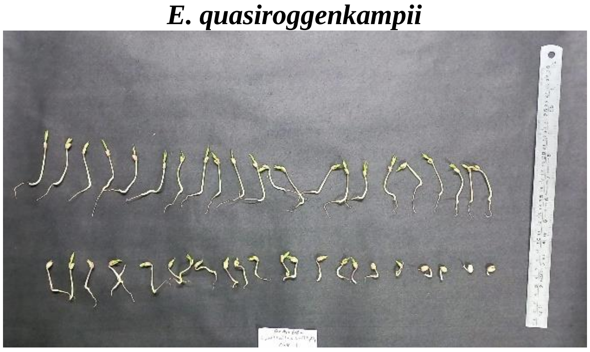
Values in the parentheses are standard errors and parameters were recorded at 8 DAS.
Values superscribed by the same alphabet are not significantly different according to Tukey test ($P< 0.05$).

Control (40mM NaCl + 6.5 mM H_3BO_3)

E. quasiroggenkampii



C. pseudoceanisediminis



E. quasiroggenkampii* and *C. pseudoceanisediminis

Figure 1: Seed germination trials of Green gram (Sindhu) NVL605 variety with *E. quasiroggenkampii* and *C. pseudoceanisediminis* under stressed conditions of salt and boron (40mM NaCl + 6.5 mM H_3BO_3)

Control (40mM NaCl + 7.5 mM H_3BO_3) ***E. quasiroggenkampii***

C. pseudoceanisediminis ***E. quasiroggenkampii + C. pseudoceanisediminis***

Figure 2: Seed germination trials of Green gram (Sindhu) NVL605 variety with *E. quasiroggenkampii* and *C. pseudoceanisediminis* under stressed conditions of salt and boron (40mM NaCl + 7.5 mM H_3BO_3).

To solve this issue, bacterial isolates that produce ACCD may be a viable approach to promise crop survival and increase output under such conditions[37]. There are reports on ACCD-producing PGPB that enhanced survival and tolerance of *Vigna radiata* under several stressed conditions. The consortia of *Aneurinibacillus aneurinilyticus* strain and *Paenibacillus* strain have significantly enhanced percent germination, root/shoot length, fresh and dry weight of biomass, relative water content (RWC), chlorophyll content as well as osmoprotectants (proline, total soluble sugar) levels in common bean seedlings under saline stress conditions[16].

Pseudomonas sp. PGR-11 has augmented the productivity of *V. radiata* (L.) even in pesticide-stressed soils[2]. ACCD activity of *Bradyrhizobium* sp. SUTN9-2 has enhanced nodulation in the early stage of plant growth, maintained nitrogen fixation and increased the plant biomass under drought conditions[38]. Similarly, *Enterobacter* sp./*L. adecarboxylata* PAB19 is also reported to alleviate the water stress of *V. radiata* by improving the biological and biochemical features under water-deficit conditions[1]. Three strains of *Pseudomonas* have effectively alleviated the rooting inhibition of mung bean cuttings under saline-alkali stress by reducing the production of ethylene[25]. *Bacillus subtilis*, *Rhodococcus* sp. and *Bacillus albus* have mitigated NaCl stress, mobilized nutrients and Iron (Fe) in *Vigna spp* (Mung bean and Black gram)[6].

Another study on co-inoculation of halo-tolerant *Pseudomonas fluorescens* and *Enterococcus hirae* with ACCD activity has been shown to induce salt tolerance and improvement in the productivity of *Vigna radiata*[24]. Even there is a report to reduce the deleterious effects of ultraviolet B (UVB) radiation on *Vigna radiata* seedlings by methylotrophic bacteria having ACCD activity[14].

In addition to ACCD activity, bacterial strains having the ability to fix atmospheric nitrogen, producing indole-acetic acid (IAA), siderophore, ammonia and hydrogen cyanide (HCN), solubilization of phosphorous, potash and zinc can successfully colonize the roots of plants and can offer protection against stress[31]. These are called multifarious PGPBs that can enhance plant growth, development and improvement in yield under different biotic and abiotic stress. Five bacterial strains of the genus *Pseudomonas* that were drought tolerant and could produce IAA, ACCD enzyme and siderophore showed the possible ability to relieve drought stress in *Vigna radiata* and improve grain yield significantly[46].

Multifarious PGPB *Pantoea agglomerans* and *Pseudomonas fragi* are reported to develop root systems in French bean growth by increasing zinc content when applied alone and in consortia under salinity stress[17]. IAA synthesis is a key mechanism in plant growth as it helps in root development and nutrients uptake, coordinates nitrogen demands and enhances seed and tuber germination. It also increases the development rate of xylem and root, helps in plant cell division and differentiation[12].

According to reports, IAA producing isolates the more potent to promote plant growth and if they have ACCD activity, it will complement to survival of the plant even under stressed environmental conditions[42]. Both isolates have shown the production of IAA.

However, only *Enterobacter quasiroggenkampii* can show ACCD activity, despite *Cytobacillus pseudoceanisediminis* is selected for study as being Gram-positive and IAA producer amongst all isolates. As per many of the published reports, PGPBs are predominantly Gram-negative[13]. *Enterobacter quasiroggenkampii* is a newly identified strain of the Enterobacter genus which was initially identified as *Enterobacter cloacae*[48]. Even *Cytobacillus pseudoceanisediminis* is also a novel species[44] which is not reported for PGP characters. Considering those reports and the ACCD ability of the isolates, their PGP characterization and effect on the growth of *Vigna radiata* was assessed.

Though phosphorus, potash and zinc are vital micronutrients in plant metabolism but they are also limiting factors for plant productivity[19]. Bacteria that solubilize potassium, can improve the growth and productivity of plants grown under salt-affected conditions by breaking down the complex potash compounds into simpler ones and making them available to plants[30]. Both the isolates were able to solubilize phosphate and potash with an almost equal solubilization index, but zinc was only solubilized by *Enterobacter quasiroggenkampii*. Nitrogen (N_2) is an essential nutrient of many important organic compounds in plants and significantly impacts plant life activities, crop yield, quality and nutrient accumulation[15].

Enterobacter quasiroggenkampii could fix nitrogen on both Norris and Ashbys media, while *Cytobacillus pseudoceanisediminis* fixed nitrogen only on the Norris nitrogen free medium. Probably this is because *Cytobacilli* are generally unable to utilize mannitol sugar present in the medium. Under certain conditions, the target PGPB synthesizes and releases siderophores, which increases and controls the bioavailability of Iron (Fe) in the plants. Siderophores exhibit a strong affinity towards Fe (III) and are small molecules (500–1500 Da), that inhibit some bacteria and fungi that are harmful to plants[45]. Similarly, bacteria that produce the enzyme chitinase also offer protection against fungal phytopathogens[47].

Both *E. quasiroggenkampii* and *C. pseudoceanisediminis* demonstrated their potential as biocontrol agents by producing siderophores and chitinase. However, *E. quasiroggenkampii* is superior to *C. pseudoceanisediminis* based on chitin hydrolysis index. Both *E. quasiroggenkampii* and *C. pseudoceanisediminis* could tolerate 8 % NaCl concentration showing moderate halophilic nature.

Also, both have ability to grow up to 75 mM concentration which is much more toxic to all types of plants. Many crops including *Vigna radiata* are sensitive to boron not beyond 7.5 mM H_3BO_3 concentration[5]. *E. quasiroggenkampii* is mesophilic and could grow up to 40^0C temperature and *C. pseudoceanisediminis* is mild thermophilic and could grow at 42.5^0C. All these tolerance assay results ensure that selected isolates can survive in fluctuating adverse environmental conditions.

The present reports on trials of isolates with *Vigna radiata* seeds are encouraging in the solution of salt and boron issues in agriculture. The plants inoculated with the *E. quasiroggenkampii* as being ACCD producer exhibited a significant rise in shoot length and vigor index solely and in combination with *C. pseudoceanisediminis* under both stress conditions.

These findings emphasize the beneficial effects of ACC deaminase in *Vigna radiata* growing under salt and boron stress. However, there were no significant beneficial effects on lateral root formation and the root length of *Vigna radiata* due to B toxicity. In the case of wheat and barley due to excessive boron, general root weakness and decreased growth of lateral roots are reported when grown hydroponically[5]. The chlorophyll content is an important metric to measure crop growth.

The health and growth status of crop plants are reflected in the amount of chlorophyll present[41]. In both stressful situations, the total Chl content was more than that of the control which highlights the beneficial effect of isolates on the health status of *Vigna radiata*. Vigour of seedlings is an agronomical trait and sign of assured seed germination, seedling growth and tolerance to adverse climatic factors[27]. The vigour index of plants inoculated with *E. quasiroggenkampii* was higher than uninoculated plants suggesting improved growth quality of plants with improved seedlings' tolerance to salt and boron stress.

Conclusion

Numerous genera are reported like *Agrobacterium, Arthrobacter, Azotobacter, Azospirillum, Bacillus, Burkholderia, Caulobacter, Chromobacterium, Erwinia, Flavobacterium, Micrococcous, Pseudomonas and Serratia* as PGPB genera that have several similar species. However, many studies also report on the genera within the family *Enterobcteriacae* as members of PGPB including *Citrobacter, Enterobacter, Erwinia, Klebsiella, Kluyvera, Pantoea and Serratia*, although some of these genera also have reported plant pathogens. *Enterobacter cloacae* is a species that is well-reported for PGP activities. This study revealed that the two isolates were multifarious, salt-tolerant and boron-tolerant PGPB, highlighting the importance of ACCD activity for host plants to survive under salt and boron stress.

In the green gram plant model, ACCD-producing *E. quasiroggenkampii* is more advantageous than the non-ACCD producer *C. pseudoceanisediminis* under salt and boron circumstances. Results with multifarious *E. quasiroggenkampii* are encouraging and have demonstrated stress protection to the plant under study. However, *E.*

quasiroggenkampii could prove to be a sustainable option for plant growth in agriculture when exposed to abiotic stressors like boron and salt.

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