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Evaluation of plant growth promoting activity of root nodule bacteria isolated from *Vigna trilobata* (L.) Verdc

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Abstract
Root nodule Bacteria may exhibit plant growth-promoting activity, which can enhance plant growth and resilience to environmental stresses. A total 34 bacterial isolates were purified from root nodule of *V. trilobata*. These isolates were further investigated for their plant growth-promoting activity, such as phosphate solubilization, phytase activity, IAA production, chitinase activity and HCN production. Five isolates; VT3, VT10, VT15, VT11 and VT23 showed positive phosphate solubilization while VT1, VT16, VT15 and VT23 showed Phytase activity. All the isolates showed IAA production. Chitinase activity was present in only two isolates; VT11 and VT19. Isolate VT11 exhibits three PGP activities i.e., phosphate solubilization, IAA production, chitinase activity.

Keywords: PGP, *Vigna trilobata*, root nodule bacteria

Introduction
Rhizobia are known to promote the growth of legumes through biological nitrogen fixation. Moreover, free living and endophytic rhizobia may act as PGPR for economically important legume and non-legume crops (Biswas *et al.*, 2000) [7]. Bacteria may promote plant growth through mechanisms including phosphate solubilization (Chamkhi *et al.*, 2023) [9], IAA production (Wang *et al.*, 2020) [35], ACC deaminase activity (Chaudhary & Sindhu, 2017) [11], production of siderophore (Joshi *et al.*, 2008) [20], lytic enzyme and biocontrol activities (Avis *et al.*, 2008) [5]. Colonization and survival of *Bradyrhizobium* and *Rhizobium* in rhizosphere of non-legumes were reported in many studies (Chaintreuil *et al.*, 2000; Hilali *et al.*, 2001; Yanni *et al.*, 2001; Lupwayi *et al.*, 2004) [8, 18, 24, 37]. Number of species of rhizobia genera including *Rhizobium, Bradyrhizobium, Ensifer, Mesorhizobium etc.* can produce phytohormones, siderophores, HCN; can solubilize phosphates, thereby improving plant nutrition (Charest *et al.*, 2005) [10]. Nascimento *et al.*, (2012) [27] reported enhanced growth promotion activity of *Mesorhizobium* strain in chickpea through expression of exogenous ACC deaminase gene. In the current study, root nodule bacteria isolated from *Vigna trilobata* were used to characterize for their potential for plant growth promotion.

Material and methods
Isolation of bacterial isolates
Bacteria were isolated from excavated root nodules by method described in Vincent (1970) [34]. Nodules were surface sterilized using 1.0% (w/v) NaOCl for 5 min and the suspension of crushed nodule was streaked on CR-YEMA medium (Yeast Extract Mannitol Agar supplemented with 25.0 µg/ml Congo red dye) for the isolation of bacteria.

Phosphate solubilization
Pikovskaya's (PVK) agar medium having tri-calcium phosphate as substrate was used to determine the ability of bacteria to solubilize phosphate (Pikovskaya, 1948) [28]. Spot inoculation of fresh activated bacterial strain was done on PVK agar medium under aseptic condition and incubated for 3-5 days. Formation of clear zone around the colonies due to solubilization of phosphate was considered as positive result.

Phytase activity
Phytase is phosphatase enzyme that catalyzes the hydrolysis of phytic acid to myo-inositol and phosphoric acid (usable form of inorganic phosphorus). Production of phytase by bacterial strains were screened by using phytase agar medium given by Howson and Davis (1983) [19]. The clear zone of phytate hydrolysis around the bacterial colonies were recorded as positive result.

IAA production
Screening of IAA producing root nodule bacteria were done using method given by Gordon and Weber (1951) [16]. YEM broth with 1 mM L-tryptophan was used for bacterial inoculation and incubated in complete dark at 28±2 °C and 120 rpm for 10 days. Bacterial cells were pellet down by centrifuge at 10,000 rpm for 15min. Two drops of ortho-phosphoric acid and 4 ml of the Salkowski reagent (1 ml 0.5 M $FeCl_3$ solution in 50 ml of 35% of perchloric acid) was added to 2 ml of the supernatant. Development of pink colour recorded as positive results for IAA production. Absorbance of pink color was read at 530 nm with the help of spectrophotometer.

Chitinase activity and HCN production
Chitinases are the group of enzymes that decomposes chitin, a homo polysaccharide made up of monomers of N-acetyl glucosamine connected by glycoside β-(1-4) bonds. Chitinase activity was screened by using colloidal chitin agar medium (Kim *et al.*, 2003) [21]. Bacterial isolates were spot inoculated and kept for 48-72 h at 28±2 °C. The formation of clear zone around the colonies with gram's iodine solution were recorded as positive result for presence of extracellular chitinases. The production of volatile HCN by bacteria were detected by the method given by Bakker and Schipper, 1987 [6] using nutrient agar slants supplemented with 4.4 gm/l glycine (0.44%).

Results and discussion

There is plethora of reports on beneficial effect of rhizobia to promote the growth of legumes through fixing biological nitrogen. Number of species of rhizobia including *Rhizobium, Bradyrhizobium, Ensifer, Mesorhizobium etc.* can produce phytohormones, siderophores, HCN; can solubilize phosphates, thereby improves plant nutrition (Charest *et al.*, 2005, Korir *et al.*, 2017; Wang *et al.*, 2020) [10, 22, 35].

The P solubilizing bacteria dissolve the soil P by lowering the pH of rhizosphere through production of low molecular weight organic acids (Deubel, 2000) [13], in addition to. Strains of *Rhizobium* and *Bradyrhizobium* with phosphate solubilizing activity to solubilize inorganic phosphate have been reported (Rodríguez & Fraga, 1999; Korir *et al.*, 2017) [22, 30]. Rivas *et al.*, (2007) [29] reported chickpea microsymbiont *Mesorhizobium ciceri* and *Mesorhizobium mediterraneum* as a good phosphate solubilizers. Alikhani *et al.*, (2006) [3] also reported 44% of isolated strains including *Bradyrhizobium*, *Mesorhizobium* and *Ensifer* were able to solubilize phosphate. In present investigation, only five isolates showed phosphate solubilization on Pikovskaya's agar medium (Table 1). Three strains VT15, VT11, VT23 showed high phosphate solubilization while two strains, *Ensifer* strain VT3 and VT10 showed low phosphate solubilization. Phytases are group of enzymes capable of releasing phosphate from phytate, organic form of phosphate. VT1, VT15, VT23 and VT16 showed phytase activity (Table 1.).

Table 1: Plant growth promoting activities of RNB strains isolated from *V. trilobata*

PGP activity	Low	High
Phosphate solubilization	VT3, VT10	VT15, VT11, VT23
Phytase activity	VT15	VT1, VT23, VT16,
IAA production	VT2, VT3, VT5, VT6, VT9, VT10, VT11, VT13, VT14, VT17, VT18, VT21, VT22, VT29, VT30, VT31, VT33, VT34	VT1, VT4, VT7, VT8, VT15, VT16, VT19, VT20, VT23, VT24, VT25, VT26, VT28, VT12, VT27, VT32
Chitinase activity	VT11	VT19
HCN production	-	-

Indole 3-acetic acid (IAA) production is considered as one of the key mechanisms of PGP (Lee *et al.*, 2006) [23]. In plants, IAA controls several physiological processes including, root initiation, cell enlargement and division, phototropism, and apical dominance, (Wulandari *et al.*, 2024) [36]. IAA production by symbiotic nitrogen fixing rhizobia strains is reported by many workers (Arora *et al.*, 2001; Ghosh & Basu, 2002; Mandal *et al.*, 2007; Sridevi & Mallaiah, 2007, Gallarato *et al.*, 2015; Ahmed *et al.*, 2021) [2, 4, 14, 15, 25, 33]. All the RNB strains isolated in the present study were found positive for production of IAA but amount varied from low to high concentration.

Chitinase enzyme is a cell wall hydrolytic enzyme that have role in controlling fungi possessing chitin as cell wall component. Mehboob *et al.*, (2011) [26] found that strains of *Mesorhizobium* and *Rhizobium* isolated from root nodules of chickpea, mung bean and lentil have chitinase activity. In the present study, only two strains; VT11, VT19 showed chitinase activity.

The HCN production by rhizospheric bacteria is a biocontrol property (Heydari *et al.*, 2008; Ryall *et al.*, 2009; Schippers *et al.*, 1990) [17, 31, 32]. Production of HCN is a common in *Pseudomonas* and *Bacillus* (Ahmad *et al.*, 2008; Charest *et al.*, 2005) [1, 10]. In the present study, none of bacterial strains was found positive for HCN production. Similarly, Deshwal *et al.*, (2003) [12] also observed that HCN production in rhizobia is very rare.

Conclusion

Rhizobia exhibit significant PGPR activity through various mechanisms, including phytohormone production, nutrient solubilization, and stress mitigation. Their ability to enhance plant growth and resilience makes them valuable components of sustainable agricultural practices. In the present study, isolate VT11 exhibits three PGP activities i.e., phosphate solubilization, IAA production, chitinase activity, which makes it a potent PGPR. The co-inoculation of rhizobia with other PGPR can further amplify these benefits, leading to improved crop yields and soil health.

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