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Estimation of Plant Growth Promoting Traits of Bacterial Endophytes Isolated from Potato Plants

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ABSTRACT: Sustainable agriculture is the need of the hour and endophytes offer an exciting prospect towards this. Many biotic and abiotic stresses hinder the plant growth ultimately resulting in yield loss. In the present study, the bacterial endophytes were isolated and were evaluated for their plant growth promotional (PGP) activities. Ten better performing isolates were selected among the 62 bacterial endophytes isolated from the potato plants. Later, these 10 isolates (PEL-4, PEL-5, PEL-6, PEL-8, PES-5, PER-6, PER-10, PEL-13, PEL-20 and PEL-22) were subjected to both qualitative and quantitative estimation for various PGP traits under *in vitro* conditions. The results showed that the strains PEL-4, PES-5, PEL-22 were superior among all the isolates. The isolate PEL-22 was the most efficient isolate which showed highest per cent of siderophore production (80.88 %), phosphate solubilisation (35.17 µg/ml) and potassium solubilisation (3.67 cm). Only 3 isolates, PEL-4, PES-5 and PEL-22 were tested positive for HCN production and 4 isolates were positive for ammonia production and these three isolates were observed to be able to produce PGP traits encouraging plant growth and yield increase.

Keywords: Bacterial endophytes, Sustainable agriculture, Plant growth promotional traits.

INTRODUCTION

Endophytes are the microorganisms (bacteria or fungi), present in the plants for the whole or a part of their life cycle, residing inter and/or intracellular healthy tissues of the host plant, without causing any noticeable disease symptoms (Gaiero *et al.,* 2013; Pandey *et al*., 2016). The term 'Endophyte' is derived from the Greek words 'endon' (within) and 'phyte' (plant). Off-late, endophytes have been gaining importance to combat the various biotic and abiotic stresses that cause damage to the crops by various means.

The extensive use of chemicals for the cultivation of crops has been causing many adverse effects on the human health and also on the environment in the recent times. In this regard, biocontrol strategies are gaining importance as a remedy or as an alternate towards sustainable agriculture and organic farming. Biological control by microbial endophytes is the best way to control pathogens as they are inherently safe, cost effective, and environmental friendly.

Biological control by microbial endophytes is the best way to control pathogens as they are inherently safe, cost-effective, environmental friendly and also sustainable (Rabiey *et al.,* 2019). Therefore, the plant-endophyte association improves plant health *via* several mechanisms and potentially contributes to the resistance of the host plants against numerous microorganisms that cause disease (Eljounaidi *et al.,* 2016; Malhadas *et al.,* 2017). Endophytic bacteria, which have been shown to be plant growth-promoting or pathogen-suppressing or to activate plant defence systems can benefit plants through enhanced resistance to biotic and abiotic stresses and plant growth promotion. Endophytes perform various activities such production of siderophores, phytohormones secretion, nutrient assimilation like phosphate and potassium solubilisation, HCN and ammonia production, *etc.*, which not only help the plants for their growth and development but also help them overcome a various range of biotic stresses by combating disease causing pathogens.

Bacterial endophytes provide additional mechanisms like niche exclusion, production of novel secondary metabolites, direct antagonism inside plants, barrier effects for vascular pathogens, *etc.* than rhizospheric microorganisms (Rosenblueth and Martinez-Romero 2006). All such effects make endophytes a suitable candidate to be used as biological inoculant in agriculture production system for biological control. Effect of endophytes is combined effect of interaction of microorganism and its host plant (Brader *et al.,* 2014); therefore, it could be more suitable for sustainably enhancing crop production. In the present study, the bacterial endophytes were evaluated for their plant growth promoting **(**PGP) activities.

MATERIALS AND METHOD

Bacterial cultures: Ten bacterial endophytes *viz.*, PEL-4, PEL-5, PEL-6, PEL-8, PES-5, PER-6, PER-10, PEL-13, PEL-20 and PEL-22 were used in this study which were previously screened for *in vitro* antifungal activity against early blight causing pathogen *Alternaria solani* by dual culture method (Srikanth *et al.,* 2023). Bacterial endophytes were cultured on nutrient agar media.

Screening of the endophytic isolates for plant growth promoting traits

Siderophore production. Chrome azurol's agar was used to estimate the production of siderophore. One single colony of culture was spot inoculated on the CAS agar plates and incubated at 30 °C for 3-4 days. Formation orange halo zone around the colony is indicated as positive for siderophore production. Nutrient agar was prepared, autoclaved and the 20 ml of CAS dye (Chromoazural) was prepared (Schwyn and Neilands 1987).

Quantification of siderophore production. The endophytic bacterial isolates were tested for their ability to produce siderophore quantification. The supernatant was extracted from 3 days old culture grown in tryptic Soya Broth. One ml of culture was centrifuged at 10,000 rpm for 10 minutes and 100 µL of supernatant was inoculated in a microcentrifuge tubes to which 100 µL of universal Chrome Azurol S (CAS) reagent was added as described by Schwyn and Neilands (1987) to detect the siderophore production and kept under room temperature for 1 h. The absorbance was recorded at 630 nm by using a UV visible spectrophotometer (Thermo scientific, Biomate 3S, China) against a reference consisting of uninoculated broth. The percent siderophore units calculated by using formula below

% Siderophore units = (Ar630 nm - As630 nm) / Ar630 nm × 100

Where, Ar = Absorbance of reference at 630 nm (CAS reagent)

As = Absorbance of sample at 630 nm.

Phosphate solubilization assay. Phosphate solubilizing ability of the endophytic bacterial isolates was analyzed on Pikovskaya's (PVK) (Pikovskaya, 1948) media plates. PVK containing five gram of tri-calcium phosphate (TCP) served as sole phosphorus source (Surange *et al*., 1997; Mehta and Nautiyal, 2001). The growth of bacterial isolates was analyzed by spot inoculation of cultures on the media and incubated at 30 °C for 7 days. The ability of the bacteria to solubilize insoluble phosphorus and form clear halozones around them was considered as positive result for their phosphate solubilization potential.

***In vitro* quantification of phosphate solubilization by the endophytic bacterial isolates.** The isolated bacterial culture was grown in nutrient broth and 1 ml of the actively grown culture was inoculated to the 100 ml of NBRIP medium with tri-calcium phosphate as a phosphate source and incubated at 28 °C for seven days. After seven days of incubation the pH of the medium was recorded and centrifuged at 10000 rpm for 5 minutes. To estimate the soluble phosphorus content in the medium 0.5 ml supernatant was taken and 1-2 drops of p-nitrophenol (0.25 %) was added as an indicator followed by addition of 5 N HCl drop wise to neutralize the colour. The above solution was diluted with 40 ml of double distilled water and 8 ml of ammonium paramolybdate-ascorbic acid reagent was added to the solution and incubated at room temperature for 20 minutes. The final volume of the solution was made up to 50 ml with double distilled water. The absorbance was read at 880 nm by using a UV visible spectrophotometer (Thermo scientific, Biomate 3S, China) (Murphy and Riley 1962).

Potassium solubilisation. The ability of potassium solubilisation by fungal endophytes was determined by growing on Aleksandrow media (Hu *et al*., 2006). Freshly grown 5 mm fungal mycelia was transferred on plates containing Aleksandrow medium (Appendix II) incubated at 30 °C for 7 days. After incubation, the clear zones formed around the colonies were measured.

HCN production test. Hydrogen cyanide production was detected as described by Bakker and Schippers (1987). Petri plates containing 10 per cent trypticase soya agar supplemented with 4.4 g of glycine per litre were inoculated with the bacterial endophytes and inverted with a lid containing filter paper, impregnated with 0.5 per cent picric acid and two per cent sodium carbonate, over each petri plate. The plates were incubated at 28 °C for three to five days. A change in colour of the filter paper from yellow to orange-brown on the filter paper indicated cyanide production.

RESULTS AND DISCUSSION

The ten isolates were screened for the various plant growth promotional traits such as siderophore production, ammonia production, HCN production, phosphate solubilisation and potassium solubilisation. The clear zone around the culture was taken as positive and the plates without the zones were marked as negative in case of all these tests (Fig. 1).

Siderophore production. The isolates PEL-4, PES-5 and PEL-22 showed maximum inhibition zone whereas isolates PER-6 and PER-10 showed moderate levels. Two isolates *viz.,* PEL-5 and PEL-6 were observed to be negative for siderophores as there was no clear zone observed in the plates (Table 1). The isolate PEL-22 produced more amounts of siderophores (80.88 %) among the ten isolates followed by PES-5 (74.02 %) and PEL-4 (72.71 %) respectively (Fig. 2). Siderophores are small molecules that have a strong affinity for chelating iron. They solubilize iron and transfer it into bacterial cells. An important mineral, iron, can be sequestered by particular endophytic bacterial siderophores, making it unavailable to harmful disease causing microbes and making it available to plants under iron-scarce conditions, which can impact the virulence of the pathogens. To aid plant growth when iron is limited, *Bacillus* sp. can release iron chelators and siderophores (Lopes *et al.,* 2018). Similar results were reported by Shetty e*t al.* (2023), while working under drought stress.

HCN production. In the same way, for ammonia production, five isolates (PEL-4, PEL-5, PES-5, PEL-20 and PEL-22) showed positive result and another five

isolates (PEL-6, PEL-8, PER-6, PER-10 and PEL-13) showed negative result (Table 1). Only four isolates (PEL-4, PES-5, PER-10 and PEL-22) were noted to be positive for HCN production and all the other six isolates were observed to be negative as the colour of the filter paper did not turn to brown due to the absence of HCN production (Table 1). Hydrocyanic acid (HCN) produced by bacterial endophytes serves as a pathogen defence mechanism for plants, induces resistance. This volatile substance prevents electron transfers, impairs the energy supply to cells, and eventually kills infections (Aarab *et al.,* 2015). Additionally, because to the sensitivity of heme groups in the target eukaryotic cells, HCN possesses broad-spectrum toxicity towards fungi, nematodes, insects, and plants (Zdor, 2015).

Phosphate and potassium solubilization. In the case of phosphate solubilization, isolates PEL-5 and PEL-20 exhibited negative result and all the other isolates were positive. Maximum zone of inhibition was seen in the isolates PEL-4, PES-5 and PEL-22 (Table 1). The maximum phosphate solubilisation was found to be in the isolate PEL-22 with 35.17 µG/ml and was followed by PEL-4 (32.28 µG/ml) and PES-5 (27.26 µG/ml). The isolate PEL-20 produced lowest amount of solubilised phosphate of 1.01 µG/ml (Fig. 3).

For the potassium solubilisation, only PEL-22 showed maximum zone in the petri plates whereas the six isolates (PEL-4, PEL-5, PEL-6, PEL-8, PES-5 and PER-6) produced moderate to less zones in the plates. The isolates PER-10, PEL-13 and PEL-20 didn't produce any zone and were noted as negative (Table 1). The bacterial endophyte PEL-22 produced the largest zone of solubilisation when tested for potassium in the petriplate with a diameter of 3.67 cm, which was proceeded by PES-5 (3.00 cm) and PEL-4 (2.53 cm) (Fig. 4).

Plants, for their growth require the macronutrients like phosphorus and potassium. The creation of cells, the manufacture of enzymes and the synthesis of protein, cellulose and vitamins all require potassium in plants. Abiotic and biotic stress can both be resisted by plants with the help of potassium (Bashir *et al.,* 2017). Similarly, for all metabolic processes in living cells, phosphorus serves as an essential universal fuel (Viruel *et al.,* 2014).

The intimate relationship between an endophyte and a plant enables the endophyte to manufacture bioactive substances through a variety of plant processes and pathways, which is favourable for the plant's growth and defence. The host plant's growth is influenced favourably by siderophore, HCN, hydrolyzing enzymes produced by endophytes (Hassan., 2017). Our findings were substantiated by the presence of endophytic plant growth-promoting bacteria from a variety of crops that are capable of nitrogen fixation (Muangthong *et al.,* 2015), PO_4 solubilization (Otieno *et al.,* 2015; Ribeiro *et al.,* 2018), and K solubilization (Yuan *et al.,* 2015) from different plant parts.

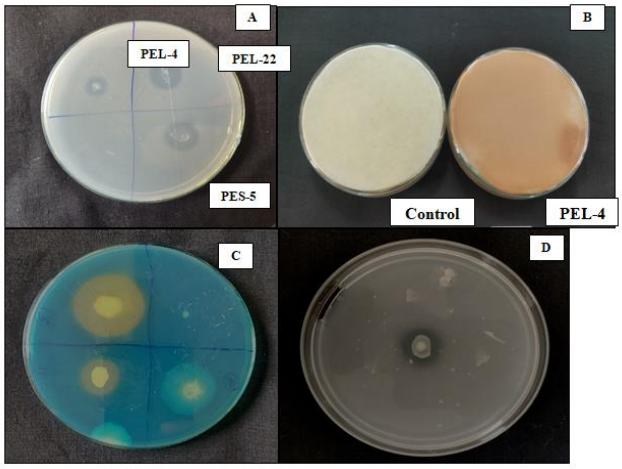

Fig. 1. Qualitative PGP traits estimation of bacteria endophytes (A) K solubilisation, (B) HCN production, (C) Siderophore production and (D) PO_4^{2-} - Solubilisation; P- plant, E- Endophyte, L-Leaf, S-Stem.

Table 1: Qualitative screening of plant growth promoting traits of bacterial endophytes.

Sr. No.	Isolate code	Siderophore	Ammonia	HCN	PO_4^{2-} solubilization	K solubilization
1.	PEL-4	+	+	+	+	+
2.	PEL-5	-	+	-	-	+
3.	PEL-6	-	-	-	+	+
4.	PEL-8	+	-	-	+	+
5.	PES-5	+	+	+	+	+
6.	PER-6	+	-	-	+	+
7.	PER-10	+	-	+	+	-
8.	PEL-13	+	-	-	+	-
9.	PEL-20	+	+	-	-	-
10.	PEL-22	+	+	+	+	+

Note: P- plant, E-Endophyte, L-Leaf, S-Stem, R-Root

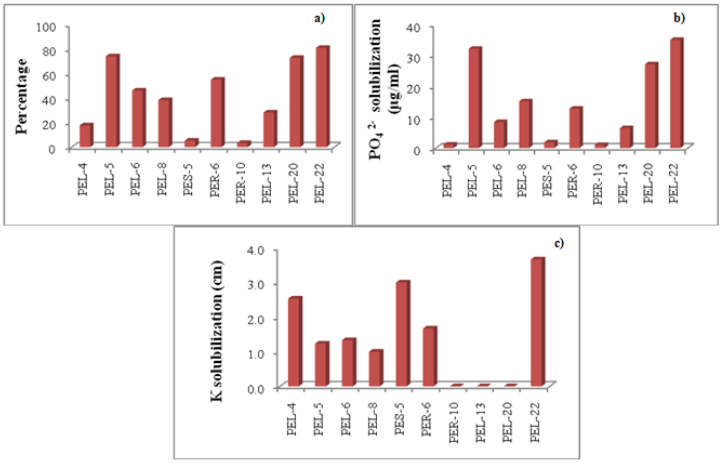

Fig. 1. Quantitative PGP traits estimation of bacteria endophytes (A) Siderophore production (B) PO_4^2 solubilisation, (C) Potassium solubilisation; P- plant, E- Endophyte, L-Leaf, S-Stem, R-Root.

CONCLUSIONS

Endophytic bacteria, which have been shown to be plant growth-promoting or pathogen-suppressing or to activate plant defence systems can benefit plants through enhanced resistance to biotic and abiotic stresses and plant growth promotion. Endophytes perform various activities such production of siderophores, phytohormones secretion, nutrient assimilation like phosphate and potassium solubilisation, HCN and ammonia production, *etc*., which not only help the plants for their growth and development but also help them overcome a various range of biotic stresses by combating disease causing pathogens. Bacterial endophytes are having novel bioactive compounds and secondary metabolites that can be potential source of antimicrobials for sustainable pest and disease management. Exploring the wide diversity of endophytes could provide new antagonists with different abilities to control plant pathogens. Incorporating a plant growth promoting (PGP) endophyte not only makes nutrient supply more affordable, but it also enhances plant health, making crop cultivation an environmentally friendly choice.

FUTURE SCOPE

This study motivates to advance investigation of selected bacterial endophytes in order to develop a bioagent promoting plant growth with applicability to multifield.

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