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EFFECT OF RHIZOBACTERIAL *STRAIN ENTEROBACTER CLOACAE* STRAIN PGLO9 ON POTATO PLANT GROWTH AND YIELD

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Abstract

To assess functional potentialities of rhizobacteria in relation to plant growth promoting activities, rhizobacterial strain *Enterobacter cloacae* PGLO9 was obtained from rhizosphere soil of potato plant (*Solanum tuberosum* L.) root. The result of *in vitro* assays showed that strain PGLO9 solubilize phosphate as well as produce siderophore and amylase. This strain having abilities for phosphate solubilization, siderophores and amylase production, were tested as bioinoculant to potato tubers via pot trail experiment. The results of inoculated potato plants showed significant increase in vegetative growth parameters such as root length, shoot length, root biomass and shoot biomass, compared with control and found better response in potato plant growth and yield against uninoculated control. Whereas potatoes harvested was healthy as well as not infected with any pathogen. Strain PGLO9 can be recommended as biofertilizer for reducing the dependence on chemical fertilizers and providing a step forward towards sustainable agriculture.

Key words: Potato, Rhizobacteria, *Enterobacter*, Root, Shoot, Biofertilizer.

Introduction

Fertilization of crop in agriculture field increases the efficiency and help in obtaining better quality of product recovery in agricultural activities (Savci, 2012). Whereas excessive use of chemical fertilizer cause various negative effect such as acidification or alkalization of the soil or soil fertility reduction, destruction of friendly insects and microorganism, crop susceptibility to disease attack, leaching, water resource pollution, thus result into irreparable damage to the complete system (Chen, 2006).

The growing concerned towards soil quality under different farming system gained attention towards environmental issue regarding degradation of soil and production sustainability (Galantini and Rosell, 2006). As environment friendly sustainable nutrient management practices is growing awareness towards maintainance and restoration of soil quality both in the short and long term usage, plant growth promoting rhizobacteria (PGPR) as effective biological technologies are being exploited for enhancing crop yield (Dinesh *et al*., 2013).

Potato is one of the chief food crops belonging to family solanaceae. As compared to any other food crop potato produce more food, edible protein and edible energy per unit area and time. Therefore, potato have major potential in eliminating malnutrition and hunger to ever increasing population provides food and nutritional security (Shankar *et al*., 2014). High demand of fertilizer for potato production and decline of soil health due to extreme use of chemicals offer large opportunities to bring into play microbial products for disease-free and healthy potato production (Kumar *et al*., 2012).

Rhizosphere soil bacteria, plays a beneficial role in plant growth promotion are termed as PGPR. They support sustainable agriculture development and protecting the environment (Das *et al*., 2013). Among the mechanism concerned in improving plant growth include atmospheric nitrogen fixation for plant use, siderophores production for iron chelation, which make iron accessible to the plant root, mineral solubilization and phytohormones synthesis (Glick, 1995).

Natural reserves of plant nutrient in the soil are present in the unavailable form to plants and only a small portion is released each year by biological activity and chemical processes (Chen, 2006). One of the bulk mineral iron present in plentiful amount on earth but exist in Fe^{3+}

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(ferric ion) form which is meagerly soluble (Hofte, 1993; Liu *et al.,* 2012). To overcome this problem, PGPR produce siderophores, which is a low molecular mass, iron binding protein having high binding affinity with ferric ion. PGPR through siderophores production promote plant growth by increasing accessible iron in root surrounding soil (Kloepper *et al*., 1980; Ma, 2005).

Whereas inspite of large reservoir of phosphorus in soil, the availability of P to the plant is low because majority of phosphorus in soil is present in insoluble form (Khan *et al.,* 2010; Bhattacharyya and Jha, 2012). Frequent application of phosphatic fertilizer in agriculture field in order to fulfill the P deficiency in soil result into fewer amount of phosphatic fertilizers absorption whereas the rest form insoluble complexes in soil (Mckenzie and Robert, 1990). Regular application of phosphate fertilizers is costly as well as undesirable for environment. This has led to search for an environmentally safe and economically reasonable choice for improving crop production in low phosphorus soil (Ahemad and Kibret, 2014).

In the present investigation effect of rhizobacteria strain *Enterobacter cloacae* strain PGLO9 which is a phosphate solubilizer as well as siderophores and amylase producer, isolated from potato rhizosphere soil has been tested for growth promoting activity on potato plant by pot trail experiment.

Materials and Methods

Microorganism and its growth promoting activity

Rhizobacteria strain PGLO9 has been used in the present study, which was isolated from potato rhizosphere soil from Lokhandi potato field Bilaspur Chhattisgarh, India and identified as *Enterobacter cloacae* species by 16S rRNA sequencing (Verma and Shahi, 2015).

Enterobacter cloacae strain PGLO9 (accession number KY492312.1) showed positive activity for phosphate solubilization [showed phosphate solubilization activity of 84.33 ± 3.188521 KH_2PO_4 (µg/ml)], siderophores production (Verma *et al*., 2018) and amylase production test. Due its enhanced growth promoting activity in maize described by Verma *et al*. (2018), in the present investigation it is tested for potato plant growth promotion.

Amylase production test

Amylase production test was performed as described by Aneja (2003). One loopful of 12 h old bacterial suspension of strain PGLO9 was streaked on starch agar medium (SAM). Plates were incubated at 37°C for 48 h. After incubation, 1% iodine solution was flood on inoculated petriplates for 5 min. Observations were recorded by presence and absence of clear zone around the rhizobacterial colony. The clear zone around the bacterial colony indicate positive test for amylase production.

Siderophore production activity

Siderophore produced by strain PGLO9 was determined by Chrome Azurol S (CAS)-shuttle assay developed by Payne (1994) adopted by Goswami *et al*. (2014). A loopful of 12 h old strain PGLO9 culture was transferred in iron-free minimal medium for siderophores production. Culture was incubated at 30°C for 48 h. Then incubated culture was centrifuged at 2700 x g for 15 min to obtain supernatant. CAS assay solution in equal proportion were transferred in supernatant of each culture, mixed properly and allowed to stand for 20 min. The reduction in the blue colour intensity was recorded at 630 nm. For the measurement, minimal media was used as blank and the % siderophore units were calculated by following formula-[(Ar-As)/Ar] × 100 = % siderophore units. Where, Ar = absorbance of reference (minimal media + CAS assay solution), As = absorbance of sample.

Pot trail experiment

i. Pot trial of strain PGLO9 on potato plants

Potato tubers of local variety were used to perform pot trial experiment which were surface sterilized by rinsing sprout potato tubers with 20% ethanol for 30 seconds followed by shaking with 1% sodium hypochlorite, 0.1% Tween-20 solution for 10 minutes and rinsed with autoclaved distill water (Naik and Chandra, 1993). Pot trial was conducted to evaluate the effectiveness of strain PGLO9 for improving growth of potato plants. It was performed in two sets. In first set (set A) as per method adopted by Firuzsalari *et al*. (2012) with few modification, sprout potato tubers were coated with 20% sugar solution as an adhesive and rolled into the suspension of rhizobacteria strain PGLO9 (10^8 cfu/ml) until uniformly coated and sown in pots containing washed and sterilized sand at the rate of 2.5 kg/pot. In second set (set B), sand was treated with 50 ml of rhizobacterial strain PGLO9 (10^8 cfu/ml). In control in place of inoculum sterilized water was used. Each treatment was replicated thrice. Potato plants were harvested after 45 days of sowing and data were recorded regarding root-shoot length and biomass, and data were statistically analysed by one way ANOVA by Tukey's multiple comparisons test using statistical analysis software programme Graph pad prism version 6.01.

ii. Pot trial of strain PGLO9 on potato yield

Data regarding potato yield was obtained by performing another pot trial experiment similarly as

described above (section i). For determining yield potato was harvested after 3 month of sowing.

Results and Discussion

Amylase production test

Amylase is a hydrolytic enzyme, amylase producing strain exhibit biocontrol pathogen property (Mota *et al*., 2017). On qualitative screening of amylase production activity, strain PGLO9 showed positive activity for amylase production test.

Quantitative test for siderophore production test

Siderophores an iron chelating compound, play an important role in the field such as agriculture, bioremediation, biosensor, and medicine (Venkat *et al*., 2017). However it also has a beneficial antagonistic role against phytopathogens (Chincholkar *et al*., 2007). Patel *et al.* (2012) recorded hydroxamate type of siderophore in the range of 11-50 mM. In the present investigation *Enterobacter cloacae* strain PGLO9 showed 15 % ± 3.24037 siderophore production activity.

Pot trial of strain PGLO9 on potato plant

On statistical analysis using one way ANOVA, statistical significant difference in root-shoot length and biomass between untreated control and treated plants (set A and set B) were recorded whereas on comparison between set A and set B, P value was found to be more than 0.05, therefore no statistical significant variation on root-shoot length and biomass were recorded between set A (rhizobacterial strain PGLO9 inoculated in seed) and set B (rhizobacterial strain PGLO9 inoculated in sand). Different scientists worked on PGPR and found positive effect of PGPR strains on plant growth. Oswald and Calvo (2009) performed pot trail experiment and found that on application of bacteria to potato plants, either to *in vitro* plantlets or to mini-tubers, which were planted in a sterilized or non-sterilized soil-sand-moss mixture recorded bacteria improved plant growth, tuber production and overall yield, and also showed more pronounced effect in increasing tuber numbers rather then tuber mean weight. Whereas Naqqash *et al.* (2016) on plant inoculation assays, of isolated rhizobacteria strain, found that different rhizobacterial strains from potato rhizosphere *Azospirillum* sp. TN10, *Agrobacterium* sp. TN14, *Pseudomonas* sp. TN36, *Enterobacter* sp. TN38 and *Rhizobium* sp. TN42 improved plant growth as compared to the uninoculated control. In the present investigation sand was selected as medium for pot trial experiment. Whereas it's an excellent medium for pot trail experiment where actual effect of bacteria can be studied, without much interference of the soil nutrient. Effect of rhizobacteria strain PGLO9 on potato growth and yield was studied by inoculating rhizobacteria strain on tuber as well as in sand and effect of *Enterobacter cloacae* strain PGLO9 in "Set A" (when strain PGLO9 was treated on tuber) on growth of potato plants was found, root length was increased at P<0.01 significant level, shoot length increased at P<0.01, root biomass increased at P<0.001, and shoot biomass increased at P<0.01 significant level as compared to untreated control. Whereas effect of *Enterobacter cloacae* strain PGLO9 in "Set B" (strain was treated on sand) on growth of potato plants was found root length was increased at P<0.05 significant level, shoot length increased at P<0.01, root biomass increased at P<0.01, and shoot biomass increased at P<0.01 significant level as compared to untreated control (Figure 1; Table 1). Phosphate solubilizing bacteria, play an essential role in plant nutrition through increasing the P uptake by the plant, their use as PGPR is an important contribution to biofertilization of agricultural crops (Rodriguez and Fraga, 1999). Hence, phosphate solubilizing, siderophores and amylase producer

Fig. 1: Effect of PGPR strain PGLO9 on potato growth. A: Strain PGLO9 inoculated on tuber, B: Strain PGLO9 inoculated on sand.

Table 1: Comparative study on growth of potato plant under treated with strain PGLO9 and untreated control.

Vegetative growth parameter	Control	A	B
Root Length (cm)	13.67 ± 0.6667	29 ± 2.687**	28.63 ± 1.691*
Shoot Length (cm)	29.33 ± 0.3512	56 ± 2.726**	54.83 ± 1.396**
Root Biomass (gm)	0.4587 ± 0.1102	4.566 ± 0.4272***	4.021 ± 0.5584**
Shoot Biomass (gm)	3.612 ± 0.3159	10.01 ± 1.5255**	11.69 ± 1.7832**

A: Rhizobacterial strain PGLO9 inoculated in tuber; B: Rhizobacterial strain PGLO9 inoculated in sand. Values represent mean ± standard error. According to one way ANOVA value represent in table are at different level of significant difference, *P=0.05; **P=0.01; ***P=0.001; ****P=0.0001; ns (not significant) =P>0.05 as compare to control.

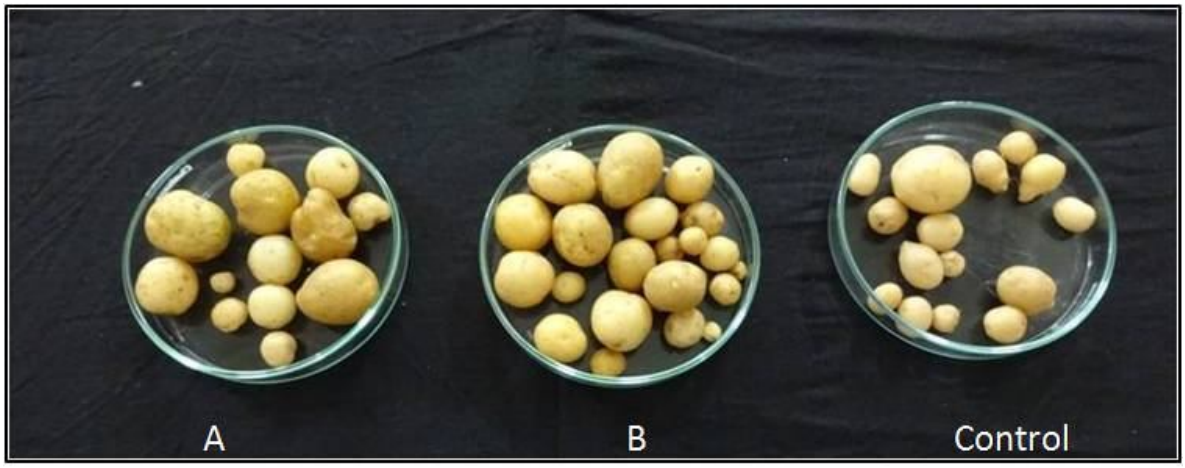

Fig. 2: Effect of PGPR strain PGLO9 on potato yield. A: Strain inoculated on tuber, B: Strain inoculated on sand

Table 2: Comparative study on potato yield with strain PGLO9 treated and untreated control.

Yield	Control	A	B
Total No. of Potato [average weight (gm)]	15 (1.12)	13 (2.9)	21 (1.92)
Potato weight (gm)	16.842	38	40.34

A: Rhizobacterial strain PGLO9 inoculated in tuber; B: rhizobacterial strain PGLO9 inoculated in sand. Values represent total no. of potato from control, set A and set B and its respective total potato weight.

strain *Enterobacter cloacae* strain PGLO9 has a significant effect on potato plant growth as compared to uninoculated control. Whereas according to Verma and Shahi (2015), *Enterobacter cloacae* strain PGLO9 also showed positive activity towards ammonia and catalase.

Pot trial of strain PGLO9 on potato for potato yield

On studying the effect of strain PGLO9 on potato yield, the total number of potato in treatment set B was higher than control, while total number of potato in treatment set A is lesser than control. Whereas total weight of potato on both the treatment set was found higher than control. On comparison between "Set A" and "Set B" total weight of potato of set B of experiment was found more as compared to set A and average weight of potato of set A found more as compared to set B. Both the mode of selected treatment shown similar effect in regard to potato yield. While treated set was recorded increased yield and potato weight in comparison to untreated control (fig. 2; table 2).

Conclusion

In the present investigation *Enterobacter cloacae* strain PGLO9 was tested for the plant growth promoting activity on potato plants. Phosphate solubilizing as well as siderophores and amylase producer *Enterobacter cloacae* strain PGLO9 helped plant in acquiring soluble form of phosphate and iron. Potato plant not only showed significant increase in root-shoot length and biomass but improvement in yield on inoculation with rhizobacteria strain PGLO9 as compared to the untreated control was also observed. PGLO9 strain can prove to be a better biofertilizer.

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