International Journal of Botany Studies
ISSN: 2455-541X
Impact Factor: RJIF 5.12
www.botanyjournals.com
Volume 3; Issue 2; March 2018; Page No. 172-175

Enterobacter cloacae strain PGLO9: Potential source of maize growth promoting rhizobacteria

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Abstract
Chemical fertilizers are being used in the agricultural field in order to increase production to fulfill the need of growing population. However chemical fertilizer has several harmful effects on environment as well as on human health. In the present investigation rhizobacteria strain *Enterobacter cloacae* strain PGLO9 has been used to observe plant (maize) growth without addition of chemical fertilizer via pot trail experiment against uninoculated control. In the experiment maize seeds variety 'JK Surabhi gold JKMH -1701 (Hybrid maize)' was treated with *Enterobacter cloacae* strain PGLO9 and vegetative growth parameter root length, shoot length, root biomass and shoot biomass was observed in comparison to untreated control. Significant increase in Maize (*Zea mays* L.) growth (root length, shoot length, root biomass and shoot biomass) was found under inoculation with strain PGLO9 as compare to uninoculated control. Therefore strain PGLO9 can prove to be an effective environment friendly microbial inoculant that can improve growth of crop and protect environment from harmful effect of chemical fertilizer.

Keywords: fertilizer, harmful, inoculant, environment, maize, root

1. Introduction
Increased crop productions largely depend on the type of fertilizers which add essential nutrient to the plants. Fertilizer application is necessary to restore crop land nutrients that have been used by earlier plant growth with the critical goal of maximizing yield efficiency with economic growth. In todays world, there is improved emphasis on the impact on soil environment due to constant use of chemical fertilizers (Rai *et al*., 2014) [18]. To lessen phosphate (P) deficiency, phosphatic fertilizers chemicals are commonly used. Though, a large fraction of the soluble forms of phosphate fertilizers is precipitated in insoluble form rapidly after application of phosphatic fertilizers and become unavailable to plants (Mahantesh and Patil, 2011) [11]. This in turn leads to a need for extreme and repeated application of soluble phosphate fertilizer, which result a serious threat to groundwater (Muleta *et al*., 2013) [13]. Excess chemical fertilizer in soil buildup heavy metals in plant system through soil, and enters the food chain. Thus, chemical fertilizer cause water, soil and air pollution (Savci, 2012) [20].
Maize is one of the most essential cereal crops after wheat and rice in world (Adjanohoun *et al*., 2011) [1]. Cultivation of maize crop is done mainly for commercial purpose with different uses like livestock feed, food, and also for raw material in ethanol, paper, starch and other industries. Maize is a chief crop in several developed and developing countries in the world, but low phophorus content is a limitation to production of maize. Shortage of phosphorus is responsible for undersized ears in maize because of crooked and missing rows as kernel twist (Masood *et al*., 2011) [12].
PGPR has huge potential to use as biofertilizing agents for a wide variety of crop plants in a broad range of climatic and edaphic condition (Reed and Glick, 2004) [19]. The microorganism colonizing rhizosphere include actinomycetes, algae, bacteria, fungi, and protozoa. Among all bacteria are the most abundant microbial present in the rhizosphere (Kaymak, 2010) [8].
The term "plant growth promoting rhizobacteria (PGPR)" was introduced by Kloepper and Schroth (Kloepper and Schroth, 1978) [10]. PGPR are not only a root association to exert valuable effect on growth of plant but also have positive effect on controlling phytopathogenic microorganisms (Kloepper *et al*., 1980; Son *et al*., 2014) [9, 21]. Therefore, PGPR is the active ingredients in biofertilizer formulation.
A key role has been played by the phosphate solubilising bacteria (PSB) in conversion of insoluble phosphate to bioavailable primary and secondary orthophosphate ions (Pal, 1998) [16] and a high fraction of PSB is found in the rhizosphere which are metabolically active than the bacteria isolated from other source (Vazquez *et al*., 2000) [25], therefore rhizosphere bacteria is of greater significance. Hence PGPR play an important role in the sustainable agriculture industry. The increasing demand for crop yield with a considerable reduction of synthetic chemical fertilizer and pesticides use is a big challenge nowadays. The utilization of PGPR has been proved to be an environment friendly sound way of increasing crop yields through either a direct or indirect mechanism by facilitating plant growth (Vejan *et al*., 2016) [23].
Very little is known about rhizobacteria associated with potato crop in India and their growth promoting effects in other plants. In the present study, growth promoting effect of phosphate solubilizing rhizobacteria strain *Enterobacter cloacae* strain PGLO9 isolated from potato rhizosphere soil was studied by pot trail experiment on maize plant.

2. Materials and Methods
Rhizobacteria and its growth promoting activity
Strain PGLO9 was isolated from rhizosphere soil of potato

from Lokhandi potato field Bilaspur Chhattisgarh, India. Strain PGLO9 showed positive activity for phosphate solubilization (Verma and Shahi, 2015) [24].

Strain PGLO9 was identified by biochemical as *Enterobacter cloacae*. Strain PGLO9 was identified by means of biochemical test and 16S rRNA sequencing. The result of partial 16S rRNA sequence alignment based on BLAST analysis, rhizobacterial strain PGLO9 was identified as *Enterobacter cloacae* species (Verma and Shahi, 2015) [24]. The sequence was deposited in the NCBI Genomic Bank nucleotide database and has been assigned accession numbers KY492312.1 (*Enterobacter cloacae* strain PGLO9).

Quantitative test for phosphate solubilization test

Quantitative test of phosphate solubilization activity was performed by colorimetric method according to Nautiyal (1999) [15] adopted by Kavamura *et al*. (2013) [7] with some modifications. 100 µl of 10^8 cells/ml (OD_{550nm} = 0.1) of *Enterobacter cloacae* strain PGLO9 were inoculated in 10 ml of NBRIP (National Botanical Research Institute's Phosphate) broth in triplicate. Tubes were incubated at l80 rpm, 28 °C for 15 days in shaking incubator, 1 ml of incubated strain PGLO9 were transferred to eppendorf tubes (2 ml) and centrifuged at 10,000 rpm for 5 minutes. Aliquots of 145 µl of supernatant of strain PGLO9 were added in 285 µl of Barton's reagent along with 570 µl of distilled water. In control in place of supernatant, uninoculated broth were used. Optical density was taken at 420 nm (SL 191 UV Double Beam Spectrophotometer, Elico) after adding the reagent. Concentration of phosphate solubilisation was estimated using KH_2PO_4 as standards.

Pot trial of potent strains on maize plant

Maize seeds variety 'JK Surabhi gold JKMH -1701 (Hybrid maize)' were used for the performing pot trail experiment. Seeds were surface-sterilized with 0.02% sodium hypochlorite for 2 minutes, and rinsed thoroughly in sterile distilled water (Gholami *et al*., 2009) [4]. Pot trail experiment was conducted to determine the effectiveness of rhizobacteria *Enterobacter cloacae* strain PGLO9 for improving growth of maize plants was performed in two sets. In first set "set A" as per method adopted by Firuzsalari *et al*. (2012) [3] with few modification, maize seeds were coated with 20% sugar solution as an adhesive and rolled into the suspension of rhizobacterial strain (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 were treated with 50 ml of rhizobacterial strain (10^8 cfu/ml). In control in place of inoculum sterilized water was used. Each treatments were replicated thrice. Maize plants were harvested after 45 days of sowing and data regarding root-shoot length and biomass were recorded. 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.

3. Results and Discussion

Quantitative test for phosphate solubilization test

The phosphorus amount is quite high in the soil but present in insoluble form and therefore unavailable to the plant. On application of soluble inorganic P to the soil as chemical fertilizer, major portion of P is immobilized soon after application and become unavailable to plants. Through solubilization and mineralization, soil microorganism play important role in releasing P from inorganic and organic forms (Hilda and Fraga, 1999) [6]. In the present investigation strain *Enterobacter cloacae* strain PGLO9 showed phosphate solubilization activity of 84.33 ± 3.188521 KH_2PO_4 (µg/ml). The inoculation of crop with selected phosphate solubilizing PGPR strain can mobilize P from insoluble phosphate present in soil in an ecofriendly way and therefore improve plant nutrition (Guinazu *et al*., 2010) [5]. Patel *et al.* (2012) [17] reported phosphate solubilization in the range of 9 - 45 µg/ml. Suresh *et al*. (2010) [22] reported phosphate solubilization of 65.10 µg/ml. In the present research phosphate solubilizing PGPR strain PGLO9 has been used to investigate growth promoting effect on maize plant which solubilize 84.33 KH_2PO_4 (µg/ml).

Pot trail Experiment

Effect of rhizobacterial strain PGLO9 on plant growth promoting activity on maize plant was studied and significant difference in vegetative growth parameter between untreated control and treated plant was confirmed by one way ANOVA analysis. On statistical analysis using one way ANOVA, statistical significant increase in root-shoot length and biomass between control and treated plants (A and B) was observed 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 was found between A (rhizobacterial strain PGLO9 inoculated in seed) and B (rhizobacterial strain PGLO9 inoculated in sand) (Table 1; Figure 1).

In the present investigation the effect of strain *Enterobacter cloacae* strain PGLO9 was studied on growth of maize plant on sterilized sand, and got significant increase in the vegetative growth parameter (root-shoot length and biomass) at various significant level as compared to untreated control.

Effect of *Enterobacter cloacae* strain PGLO9 (when strain was treated on seed "set A") on growth of maize plants was studied and found root length was increased at $P<0.01$ significant level, shoot length increased at $P<0.05$, root biomass increased at $P<0.05$, and shoot biomass increased at $P<0.01$ significant level as compared to untreated control.

In the maize pot trail two mode of inoculation of bacteria was compared, one is seed treated and another is sand treated mode of inoculation. Whereas on comparison between seed treatment and sand treatment inoculation, no significant difference was found. But significant difference was found in between untreated control and treatment set (both set A and set B).

Effect of *Enterobacter cloacae* strain PGLO9 (when strain was treated on sand "set B") on growth of maize plants was performed and recorded root length was increased at $P<0.01$ significant level, shoot length increased at $P<0.05$, root biomass increased at $P<0.05$, and shoot biomass increased at $P<0.01$ significant level as compared to untreated control.

Enterobacter as effective strain has also been reported by Dutta *et al.* (2015) [2], they found *Enterobacter* species as potent strain for the growth of tea clone TV19. *Enterobacter*

as effective rhizobacterial strain isolated from potato rhizosphere soil sample were reported by Naqqash *et al.* (2016) [14]. In the present work, *Enterobacter cloacae* strain PGLO9 was isolated from potato rhizosphere due to its potent growth promoting activity (phosphate solubilization 84.33 µg/ml) it is selected as potent strain for the study of growth promoting activity on maize plant and significant increase in root-shoot length and biomass of maize on inoculation with *Enterobacter* species was found. According to Verma and Shahi (2015) [24], *Enterobacter cloacae* strain PGLO9 also show positive activity towards ammonia, catalase, and siderophores production.

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

Vegetative growth parameter	Control	A	B
Root Length (cm)	15.74 ± 1.257	31.33 ± 2.028**	32.67 ± 1.764**
Shoot Length (cm)	19.46 ± 1.458	32 ± 1.732*	30.33 ± 2.180*
Root Biomass (gm)	0.245 ± 0.021	0.5543 ± 0.056*	0.5273 ± 0.061*
Shoot Biomass (gm)	0.503 ± 0.034	1.872 ± 0.186**	1.787 ± 0.159**

A: Rhizobacterial strain PGLO9 inoculated in seed; 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.

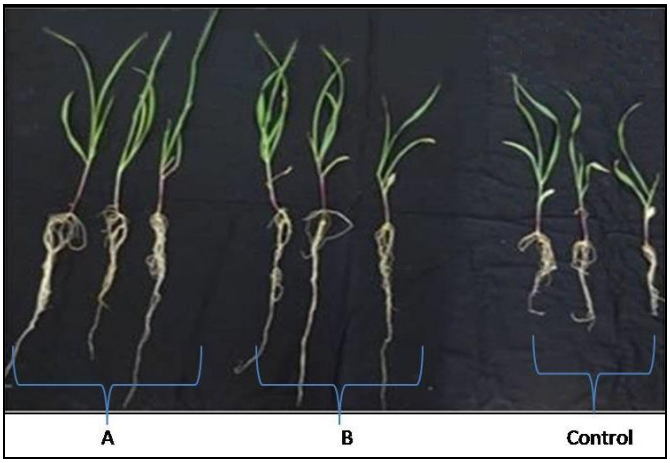

Fig 1: Effect of PGPR strain PGLO9 on maize growth A: Strain PGLO9 inoculated on seed, B: Strain PGLO9 inoculated on sand

4. Conclusions

In the present investigation *Enterobacter cloacae* strain PGLO9 was tested for the plant growth promoting activity on maize plants. Potent phosphate solubilizing strain *Enterobacter cloacae* PGLO9 helped plant in acquiring soluble form of phosphate. Maize plant showed improvement in growth and resulted into significant increase in root-shoot length and biomass on inoculation with rhizobacteria strain PGLO9 as compared to the untreated control. PGLO9 strain can prove to be a better biofertilizer.

5. Acknowledgements

We thank to the Head, Department of Botany, Guru Ghasidas Vishwavidyalaya, Bilaspur Chhattisgarh for providing the facilities, Department of Science and Technology (DST) Government of India, New Delhi for DST INSPIRE Fellowship (No. DST/INSPIRE Fellowship/2012/495). The authors also thankful to Chromous Biotech Pvt. Ltd, Bangalore, India for performing 16S rRNA sequencing.

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