Effect of coinoculation of *Mesorhizobium cicer* with PGPR on *Cicer arietinum*

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**Abstract:** In this study chlorpyrifos degrading DKC₂ and malathion degrading DKM₃, DKM₅, DKM₈, DKM₉ bacterial strain were taken. The culture DKC₂ showed siderophore production and strong plant growth promoting activity when coinoculated with *Mesorhizobium cicer* Ca181. The strain DKC₂ caused maximum gain in plants dry weight ratio 3.98 and 3.75 times when coinoculated with *Mesorhizobium* at 90 and 120 days respectively as compare to control. Thus the bacterial strain DKC₂ can be used for plant growth promotion and as a biofertilizers to provide Fe nutrition to plants. Since bacterium showing wide application in agro biotech with its pesticide degradation and plant growth promoting activity more characterization of this bacterial strain is needed for its commercialization.

**Key words:** Coinoculation, *Mesorhizobium cicer*, PGPR, *Cicer arietinum*, Plant growth

**INTRODUCTION**

Plant growth-promoting rhizobacteria (PGPR) are free-living soil-borne bacteria that colonize the rhizosphere, and when applied to seed or crops enhance the growth of plants (Kloepper 1980). PGPR enhance plant growth either by direct or indirect mechanisms (Glick 1995). Bertrand et al. (2001) identified bacteria belonging to the genera *Pseudomonas*, *Agrobacterium* and *Phyllobacterium* as the most efficient PGPR associated with canola. The different microbial populations interact with each other and with the plant- microbe interactions, which are useful, neutral or harmful, have been found to influence plant growth accordingly (Astrom et al. 1993). The detrimental environmental impact of chemical fertilizers and their rising costs, the use of PGPB as natural fertilizers is beneficial for the development of sustainable agriculture. PGPR promote plant growth by various mechanisms that includes: (i) The ability to produce indole acetic acid (Suresh et al. 2010), gibberelic acid (Mahmoud et al. 1984), cytokines (Tien et al. 1979) and ethylene (Glick 1995); (ii) symbiotic nitrogen fixation (Hayat et al. 2008a); (iii) asymbiotic nitrogen fixation (Barka et al. 2006); (iv) antagonisms against phytopathogenic microorganisms by the production of siderophores (Suresh et al. 2010), β-1,3-glucanase (Stephane et al. 2005), chitinase (Frankowski et al. 2001), and antibiotics (Nakayama et al. 1999). Bioremediation is the application of biological processes for the cleanup of hazardous chemicals present in the environment (Gianfreda and Rao 2004). It has advantages over physicochemical methods due to several merits: cost-effective, convenient, complete degradation of organic pollutants and no collateral destruction of the site material or its indigenous flora and fauna (Timmis and Pierer 1999). The widespread use of PGPR for the environmental remediation is a promising field (Lucy et al. 2004). It requires improving the effectiveness of too little amounts of external inputs by employing the best combinations of beneficial bacteria in sustainable agriculture production systems. Therefore, the present study was undertaken with the following objective: detection of siderophore production and coinoculation of PGPR strains with *Mesorhizobium* sp. *Cicer* strain Ca181in chickpea under pot house conditions

**MATERIALS AND METHODS**

**Chemicals and Microorganisms:**

The chemicals, media and reagents used for the present studies were from Hi Media Laboratories, SRL, Glaxo and E. Merck etc. The chemicals were of AR grade. Standard culture of *Mesorhizobium* sp. strain *Cicer* Ca181 used in the present studies was taken from the Department of Microbiology, CCS Haryana Agricultural University and maintained on Luria Bertani medium. Seeds of chickpea (*Cicer arietinum*) var. HC3/8618 were obtained from Pulses section, Department of Seed Technology, CCSHAU, Hisar. Different culture of PGPR (DKC₂, DKM₃, DKM₅, DKM₈ and DKM₉) used in the present studies was taken from the Department of Biotechnology, UIET, Kurukshetra University and maintained on nutrient agar medium. The bacterial culture DKC₂ was responsible for chlorpyrifos degradation with some plant growth promoting activity (IAA). The bacterial cultures (DKM₃, DKM₅ and DKM₈) were responsible to utilized of malathion as a sole carbon source with some plant growth promoting activity.

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Detection and Nature of Siderophore:
The detection of siderophore in PGPR strains was done by using the method of Schwyn and Neilands (1987). To check the nature of siderophore, the strain DKC2 was grown in culture media (Peptone 20 g, K$_2$HPO$_4$ 1.5 g, MgSO$_4$7H$_2$O 1.5 g, glycerol 10 ml in 1000 ml distilled water). After 48 h culture medium was centrifuged to obtained cell free supernatant, then takes 1 ml of supernatant and adds few drops of FeCl$_3$, OD was taken by using spectrophotometer. Cell free supernatant without FeCl$_3$ was used as a control.

Effect of PGPR Strains on Seedling Growth of Chickpea:
Healthy seeds of chickpea var. HC3/8618 were surface sterilized with acidic alcohol (H$_2$SO$_4$: ethanol, 7: 3 v/v) for 3 minutes followed by six thorough washings with sterilized water. The surface sterilized seeds were inoculated with broth cultures of PGPR strains (DKC2, DKM3, DKM5, DKM8, and DKM9) for 30 min. The inoculated seeds were germinated on plain water agar (0.8 %) at 28±1 °C. Uninoculated seeds treated with LB broth were sown on agar plates as controls. Effects of inoculation on root and shoot length were measured at 8 and 15 days of growth.

Coinoculation of PGPR Strains with Mesorhizobium sp. cicer Strain Ca181 in Chickpea Under Pot House Condition:
Chickpea var. HC3/8618 was used for symbiotic performance; healthy seeds were surface sterilized with acidic alcohol. Surface sterilized seed were inoculated with broth culture of Mesorhizobium sp. cicer stain Ca181 alone and as coinoculated by mixing with PGPR strains in a ratio of 1:1 (v/v). The 2 ml mixed inoculum was used for 15 seeds and culture was allowed to be adsorbed on the seeds for half an hour. In case of strain Ca181, 1 ml water was added to have relatively same level of inoculums. Control treatment without inoculation was also kept. Seeds sown in sterilized pot, after germination only three healthy seedlings were kept. The pots were put in pothouse under day light conditions. The plants were uprooted after 90 and 120 days to determine the plant dry weight. For preparing pot: The pot and soil was autoclaved separately at 15 lbs for 3 hr. After autoclave the soil was filled in pot up to 2/3 portion. The treatments were as following: (A) Seed (without inoculation), (B) Seed + Mesorhizobium, (C) Seed + DKC$_2$, (D) Seed + DKM$_3$, (E) Seed + DKM$_5$, (F) Seed + DKM$_8$, (G) Seed + DKM$_9$, (H) Seed + Mesorhizobium + DKC$_2$, (I) Seed + Mesorhizobium + DKM$_3$, (J) Seed + Mesorhizobium + DKM$_5$, (K) Seed + Mesorhizobium + DKM$_8$, (L) Seed + Mesorhizobium + DKM$_9$

Results:
Detection and Nature of Siderophore Production by PGPR:
Out of five, only two strains DKC2 and DKM5 were found to produce siderophore on CAS medium plates as shown in Fig. 1. The CAS assay is a functional assay based on the high affinity of siderophores for iron and is independent of their structural classification. When a siderophore removes the iron from the Fe-CAS-hexadecyltrimethylammonium bromide (HDTMA) complex, its color turns from blue to orange. The 1 ml of DKC2 cell free supernatant was added with few drops of FeCl$_3$ solution and OD was taken at different wavelength by using spectrophotometer. It was found that supernatant showing a peak at 495 nm.

Fig. 1: Zone formation by strain DKM5 and DKC2 on CAS plates.

Effect of PGPR Strains on Seedling Growth of Chickpea:
The three strains DKM$_3$, DKM$_5$ and DKM$_8$ showed stunning effect on root growth at 8 and 15 days in chickpea as shown in fig 2. Maximum stunting effect on root was shown by strain DKM$_5$ and DKM$_8$ at 8 and 15 days followed by strain DKM$_3$. A very slight stimulation on root was shown by strain DKC$_2$ & DKM$_9$. All the strain was not showing any stimulation or stunting effect on shoot growth at both 5 and 15 day of seedling.

Co Inoculation of PGPR Strains with Mesorhizobium sp. cicer Ca 181:
Seed inoculation with Mesorhizobium sp. cicer Ca 181 alone or on coinoculation with PGPR strains (DKC$_3$, DKM$_1$, DKM$_5$, DKM$_9$ and DKM$_8$) increase the plant dry weight of chickpea in comparison to uninoculated control as shown in fig 3. The plants dry weight increases by 2.06 times when inoculated with Mesorhizobium and the plants dry weight increases from 2.06 to 3.22, 3.539, 3.74, 3.84 and 3.98 times when seeds were
coinoculated with DKM₄, DKM₃, DKM₅, DKM₆, and DKC₂ respectively at 90 days and from 1.89 to 3.11, 3.30, 3.48, 3.6 and 3.75 times at 120 days as compare to control. The maximum gain in plants dry weight 3.98 and 3.75 times was found when seeds were coinoculated with DKC₂ strain and *Mesorhizobium* at 90 and 120 days respectively.

![Fig. 2: Effect of PGPR strains on seedling growth (cm) (A- root, B- shoot) under aseptic conditions.](image)

![Fig. 3: Effect of co inoculation of PGPR strains with *Mesorhizobium* on plant growth.](image)

**Discussion:**
Several studies have demonstrated the production of siderophore, other secondary metabolites and lytic enzyme production by rhizospheric bacteria were involved in the control mechanism against plant root pathogens (Nagraj kumar *et al*., 2004). Siderophore producing bacteria are good candidates for plant growth promotion, especially in neutral to alkaline soil. In our study two PGPR strains DKC₂ and DKM₅ were found to produce siderophore on CAS agar plates. The nature of siderophore produced by strain DKC₂ was catecholate in nature. The three PGPR strains (DKM₃, DKM₅, & DKM₆) showed stunning effect on root growth at 8 and 15 days in chickpea, maximum stunning effect on root was shown by strain DKM₃ and DKM₅ at 8 and 15 days followed by strain DKM₆. A very slight stimulation effect on both root & shoot was shown by strain DKC₂ & DKM₈. The root proliferation could be due to the production of antibiotic or siderophore like compound, the stunning effect could be due to the contact of bacterial cells with legume seeds or due to synthesis or secretion of excessive amount of IAA or some inhibitory agent when the bacterium was grown in synthetic medium or in root exudates of chickpea. It is also possible that phytoalexins produced by seedlings as a host defense response after inoculation of PGPR could be inhibitory for seedling growth. Similar improvement of seed germination parameters by rhizobacteria has been reported in other cereals such as sorghum (Raju *et al*., 1999), and pearl millet (Niranjan *et al*., 2004). The improvement in seed germination by PGPR was also found in work with wheat and sunflower (Shaukat *et al*., 2006). It was found that some PGPR induced increases in seed emergence, in some cases achieving increases up to 100 % greater than controls. These findings may be due to the increased synthesis of hormones like gibberellins, which would have triggered the activity of specific enzymes that promoted early germination, such as amylase, which have brought an increase in availability of starch assimilation.

In this study, it was found that seed with *Mesorhizobium sp.* cicer Ca 181 alone or on co inoculation DKC₂, DKM₃ DKM₅, DKM₆ and DKM₈ increase the plant dry weight of chickpea in comparison to uninoculated control as shown in Fig 3. The maximum gain in plants dry weight 3.98 and 3.75 times was found when seeds were coinoculated with DKC₂ strain and *Mesorhizobium* at 90 and 120 days respectively. The results are supporting the other studies on coinoculation of PGPR with rhizobia on plant growth. Coinoculation of *Pseudomonas* sp. with rhizobia has been reported to enhance nodulation, plant dry matter and grain yield in other legumes like clover (Derylo and Skorupska, 1993), pea (Bolton *et al*., 1990) and soybean (Dashti *et al*., 1996).
1998). These results suggested that PGPR strains (DKC$_2$, DKM$_3$, DKM$_5$, DKM$_8$, and DKM$_9$) acted synergistically with the *Mesorhizobium* sp. *cicer* strain Ca181 and were effective in promoting growth of chickpea. The bacterial strain DKC2 can be used for dual purpose, for plant growth promotion and chlorpyrifos degradation in soil. Since bacterium showing its pesticide degradation and plant growth promoting activity more characterization of this bacterial strain is needed for its commercialization.

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