Interaction of *Kreyellidae* sp. and plant growth promoting bacteria influences the soil characteristics and root structure of rice plants

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Abstract

Plant growth and productivity depend on the complex and dynamic interaction between the plant roots and soil microflora. At present, the research on rhizosphere associated microbes' is largely focused on bacteria and fungi; whereas the interaction of soil protists with plants and other microbes remain unexplored. The present study aimed to investigate the impact of a ciliate (*Kreyellidae* sp. C5) and two plant growth-promoting bacteria (PGPB) i.e., *Pseudomonas* sp. (Ps) and *Enterobacter* sp. (Ec), on the growth of rice plants. It was observed that the protist-PGPB interaction significantly modified the root structure leading to an enhanced outgrowth of lateral roots (272.08% – 380.41%) and seminal roots (190.40% -250.45%), in addition to an increase in the primary root length (Turkey's HSD, $p < 0.05$). The Phospholipid Fatty Acid (PLFA) analysis indicated a striking shift in the overall soil microbial communities due to the presence of a predator. The combined treatments (with C5Ps and C5Ec) further increased the Microbial Carbon Biomass (MBC) to 223.59% and 310.57% as compared to control and PGPB treatments respectively. A similar enhancement of dehydrogenase enzyme activity was observed in soil samples of rice plants on combined treatments. In contrast, the alkaline phosphatase and fluorescein diacetate enzyme activities were recorded to be more in soil samples treated with PGPB. The combined treatment of rice plants also enhanced the uptake of N and P moderately, as compared to PGPB treated plants. However, this enhancement was significant compared to control plants. The colony-forming unit (CFU) and most probable number (MPN) was found to be more in C5Ec ($131.0 \pm 3.70 \times 10^{11}$ and 5.12 ± 0.06) and C5Ps ($24.10 \pm 2.19 \times 10^{10}$ and 10.52 ± 0.39), as compared to control and PGPB treated soil samples. In conclusion, this is the first study that demonstrates significant modification of root structure and increased nutrient uptake by rice plants through interaction between *Kreyellidae* sp. and PGPB. In addition, we also report improved respiration and diverse microbial population in soil samples on combined treatment of rice plants.

Introduction

The rhizosphere is the region of soil surrounding the plant roots. It is densely populated with life forms of different domains (insects and micro-organisms), and hence considered as a hotspot for microbial interactions [1, 2]. In the soil ecosystem, plant roots provide nourishment to soil microbes by releasing root exudates. In turn, the Plant Growth-Promoting Bacteria (PGPB) in the surrounding soil stimulates plant growth through direct and/or indirect mechanisms. Direct mechanisms include nitrogen fixation, solubilization of macro- and micro-nutrients from the soil, production and/or regulation of phytohormones. The indirect mechanisms involve suppression of phytopathogens and induced systemic resistance in plants against a broad range of phytopathogens [3]. The PGPB are extremely valuable for sustainable agricultural processes. However, their survival and efficacy in soil are the most important factors for sustainable plant growth promotion.

It has been reported that the presence of protists in the rhizosphere region increases the activity and survival of PGPB in soil [4]. It is observed that selective grazing by protists induces shifts in the microbial

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 indole-3-acetic acid (IAA) producing bacteria [5] or nitrifying

bacteria [6]. Since IAA is responsible for the development of lateral root primordia and root elongation in plants, it favors plant growth promotion via enhanced nutrient uptake through increased root surface area. The stimulated nitrogenase activity of *Stenotrophomonas* sp. and *Azospirillum lipoferum* in response to ciliate predation pressure has also been reported recently [7]. In addition, the presence of protists also stimulates the biocontrol activities of certain bacteria against soil-borne plant pathogens. The increased production of siderophores [8, 9], up-regulation of cyclic lipopeptides [10] and 2, 4-DAPG production [11] by *Pseudomonas fluorescens* has also been suggested earlier in the presence of amoebae. In another study, production of secondary metabolites by *P. fluorescens* in response to grazing improved its competitive advantage over the other bacteria [12], which proved to be beneficial for plant growth and overall health. Even when grazed upon by amoebae, it was found that 2, 4-DAPG synthesis was upregulated by *P. fluorescens* [11]. This compound directly induces systemic resistance in plants [13]. In literature, most reports on the effect of protist-bacteria interaction on plant growth promotion have used amoebae as a model predatory species. The involvement of ciliate species in plant growth promotion studies is rare, except for some recent reports [14–16]. The capability of ciliate to feed on a wide variety of bacterial species depending upon size, motility, pigmentation and microcolony formation [17] has been well demonstrated in literature. However, the impact of ciliate predation on PGPB and their combined influence on plant growth has been an under-researched topic.

Therefore, the present investigation was carried out to study the impact of ciliate species (having a predation effect on PGPB) on plant attributes. For this purpose, the ciliate species was isolated from rice rhizosphere soil and two previously characterized PGPB i.e., *Pseudomonas* sp. and *Enterobacter* sp. [18, 19] were used to study their effects on rice plants. Further, the impact of bacterial and ciliate interactions on soil respiration, nutrient uptake by plants and phospholipid profiles of soil samples were also studied.

Materials And Methods

Microorganisms and culture conditions

Two previously characterized PGPB, i.e., *Pseudomonas* sp. and *Enterobacter* sp., were selected for the present study [18, 19]. Both the cultures were pre-grown in nutrient broth before performing each experiment.

A ciliate strain designated as C5 was isolated from rice rhizosphere soil sample by following a standard protocol [20]. The ciliate was separated from the soil slurry using micro capillary tubes and transferred to sterile Page's saline on a microscopic slide. After confirming the single organism under the microscope, the ciliate was transferred to Nutrient broth (1 %) in Neff's Modified Amoebae Saline (NB-NMAS) medium containing heat-killed suspension of *E. coli* as a food source. For identification, proteinase K digestion method was followed with some modifications [20] to extract the genomic DNA. The extracted DNA was used as template for 18S rDNA amplification using eukaryotic specific primers EU347F (5'-AGG GTT CGA TTC CGG AGA-3') and EU929R (5'-TTG GCA AAT GCT TTC GC-3') [21]. PCR reaction was carried out in 50 μ l reaction buffer with $MgCl_2$ (5 μ l), 0.25 mM dNTP mixture (5 μ l), 1U *Taq*

DNA polymerase (0.7 μ l), 0.1 μ M forward (2.5 μ l) and reverse primer (2.5 μ l) and 5 μ l template DNA extracted from ciliate. The thermal cycler was programmed as follows: initial incubation at 94°C for 5 min, 35 cycles of denaturation at 94°C for 45 sec, annealing at 53°C for 1 min, and extension at 72°C for 1 min, followed by final extension step of 10 min at 72°C. The sequencing of PCR products was carried out at National Collection of Industrial Microbes, Pune, India. The obtained 18S rDNA sequence was compared with 18S rDNA gene sequence available at NCBI GenBank database (<http://www.ncbi.nlm.nih.gov>) using BLASTn programme for identification of the ciliate species.

Growth Assessment Assay Of Ciliate Species With PgpB

The fresh cultures of selected PGPB were streaked (two lines) across the 0.2 X nutrient yeast extract agar plates [22]. After 3 h of incubation, 5.0 μ l (200 cysts μ l⁻¹) of ciliate were spot inoculated on one end of the streaked line. The other streaked line (of PGPB) was used as a control to compare the changes in the growth pattern. The inoculated plates were incubated at Room temperature (RT) for 5 days under dark conditions. On the other hand, the ciliate species (20,000–25,000 cysts ml⁻¹) in the broth medium were added to a 20 ml culture suspension flask and allowed to acclimatize for 3 h before the introduction of PGPB (1.1×10^7 to 2.26×10^7 CFUs ml⁻¹). The flasks were incubated at RT on a rotary shaker at 50 rpm for up to 5 days. MPN and CFU counting was done using 1 ml aliquots from each flask, for 5 days, using Neubauer's chamber under the light microscope and on nutrient agar plate respectively. Each experiment was performed in triplicates.

Impact of ciliate-PGPB interaction on plant growth

Plate assay

The effect of ciliate-PGPB interaction on the growth of rice plant was studied using plate assay method [23]. For this purpose, rice seeds (Gurjari variety) were surface sterilized in 70% ethanol for 5 min, followed by treatment with 2% v/v sodium hypochlorite for 10 min. After 8–10 washes with sterile distilled water, the seeds were transferred to a 24-well microtiter plate containing 100 μ l sterile distilled water and incubated at 20°C in the dark until germination. After 3 days, the germinated seeds were fixed on the edge of the surface of NB-NMAS agar plates [23]. This media was selected for our study since it avoids the overgrowth of bacteria (due to low concentration of nutrients) and supports the growth of protists species. To prepare the agar plates, 10 ml NB-NMAS medium was poured in sterile Petri dishes (110 $\times$ 15 mm, diameter $\times$ height) and kept at an angle of 30° until solidification. The experiments were set up for three different treatments; 1) control (without bacteria or ciliate inoculation), 2) only PGPB inoculation, and 3) PGPB plus ciliate inoculation. The interactive effect of ciliate-PGPB on early rice plant growth was determined by measuring the shoot length, primary root length and number of seminal and lateral roots of rice seedlings after 10 days of incubation.

Pot Assay

For the pot assay, the experiments were set up in plastic pots (12 inches) under greenhouse conditions. The pot experiments were conducted using loam soil collected from the agricultural fields (pH 7.2; N₂-7.3%; organic carbon-1.28%; P-403.2 mg/kg; K-288.7 mg/kg). The surface-sterilized rice seeds (as mentioned above) were exposed to five different treatments and subsequently shade dried for 2 h before sowing into the pots. The treatments were done using 24 h old bacterial culture (10⁸ CFUs ml⁻¹) and active ciliate culture (10³ ml⁻¹). The experimental setup included (1) control (without any treatment), (2) *Enterobacter* sp. (Ec) alone (3) *Pseudomonas* sp. (Ps) alone, (4) *Enterobacter* sp. + C5 (C5Ec) and (5) *Pseudomonas* sp. + C5 (C5Ps). Five replicates were used for each treatment in a completely randomized block design. The pots were watered every alternate day to retain moisture content, and they were maintained at natural day and night conditions in the greenhouse. After one month, the plants were harvested to measure root length, shoot length and dry weight.

Analysis Of Soil Samples

The bacterial communities in the soil samples were enumerated as colony-forming unit (CFU) g⁻¹ while protists were enumerated using modified Singh's most probable number (MPN) technique [24]. Total N and P content in the plant biomass were determined based on the methods described by Gebremikael [25] and Hogue [26] respectively. The Phospholipid Fatty Acid Analysis (PLFA) was undertaken at Royal Life Sciences Pvt. Ltd, Secunderabad, India [27]. The soil respiration analysis was conducted using fluorescein diacetate (FDA) hydrolysis assay of the soil samples using the method described by Adam and Duncan [28]. Dehydrogenase activity in soil was estimated using a 2-3-5-triphenyl tetrazolium chloride (TTC) reduction method [29]. The alkaline phosphatase activity of the soil samples was estimated by the method described by Bessey [30]. The microbial carbon biomass was estimated by the chloroform fumigation incubation method described by Anderson and Ingram [31]. The microbial biomass C (MBC) was calculated using the following equations

$$\text{OrganicC}(\%) = \frac{(A \times M \times 0.003)}{g} \times \frac{E}{S} \times 100$$

$$A = \{ml(HB) - ml(\text{sample})\} \times \frac{ml(UB) - ml(HB)}{ml(UB)} + \{ml(HB) - ml(HB) - ml(\text{sample})\}$$

$$\text{MBC} = \{\text{OrganicC}(\text{fumigated soil}) - \text{OrganicC}(\text{Unfumigated soil})\} \times 2.46$$

where, M = Molarity of ferrous ammonium sulphate (0.033M); G = Dry soil mass (g); E = Extraction volume (ml); S = Digest sample volume (ml); HB = Heated blank and UB = Unheated blank.

Statistical analysis

Statistical analysis of plant growth parameter, soil respiration and nutrient uptake were analyzed using one-way analysis of variance (ANOVA) in SPSS version 23.0. Comparisons between means were performed at a 5% probability level with a Tukey-test (Tukey's HSD). Principal component analysis was carried out using Minitab software to differentiate the PLFAs of the soil microbial community.

Results

Identification of the ciliate

The isolated ciliate was identified based on 18S rDNA sequencing technique. The results revealed that the strain C5 belonged to the genus *Kreyellidae* (MW274681).

Growth assessment assay with PGPB

The growth assessment assay of predator-prey interaction revealed that *Kreyellidae* sp. suppressed PGPB (*Enterobacter* sp. and *Pseudomonas* sp.) growth on NYEA (Fig. 1A). This was ascertained by visual observations under the light microscope (10X) and agar plates showing confluent growth elimination along the streaked line of test bacteria. However, in the liquid medium, the bacterial response to predation pressure was different. *Enterobacter* sp. count reduced sharply from 1.98×10^9 to 3.4×10^5 CFUs ml⁻¹ under ciliate predation pressure after 24 h, whereas *Pseudomonas* sp. count remained constant at 2.57×10^{11} CFUs ml⁻¹ for 96 h (Fig. 1B). The drastic increase in a logarithmic growth state of ciliate, in response to bacteria, was observed from 24 to 72 h. After 72 h, the growth rate reduced, and with time, the ciliate was encysted. Though the growth and encystment in response to *Pseudomonas* sp. and *Enterobacter* sp. were different, neither of the bacterial species harmed the ciliate (Fig. 1C).

Interactive effect on plant growth

The study of combined impact of both the organism on plant growth revealed more ramified root structure and increased shoot length in plate assay (Table 1). The combination of ciliate and PGPB (C5Ps and C5Ec) significantly enhanced the formation of lateral roots (272.08% and 380.41%) and seminal roots (190.40% and 250.45%). The combined treatment also increased shoot and primary root length as compared to control and bacterial treatment alone (Turkey's HSD, $P < 0.05$). A similar effect was also observed with pot studies (Table 2). A significant variation was observed in the root length on combined treatment of seeds (Fig. 2; Turkey's HSD, $P < 0.05$). Overall, it was observed that the combined treatments led to the development of significantly different root structure. There was not much variation observed in the shoot length on any of the treatments in this study. However, significant enhancement in dry weight of shoot and roots of rice plant was observed on treatment with C5Ps and C5Ec. Our results indicated that Ec significantly increases the plant growth compared to control; however, the combination of C5 + Ec had the highest impact on lateral root formation and primary root length.

Densities of bacteria and protists in soil

Enumeration of bacteria and protist from the soil sample revealed that the total number of cultivable bacteria varied depending upon the treatment (Turkey's HSD $P < 0.05$). The CFU was found to be greater in C5Ps ($24.10 \pm 2.19 \times 10^{10}$) and C5Ec ($131.0 \pm 3.70 \times 10^{11}$) treatment compared to control and PGPB treatment alone (Table 3). In case of the MPN study, the protist population was found to be higher on C5Ps (10.52 ± 0.39) and C5Ec (5.12 ± 0.06) treatment (Table 3).

Shifts in microbial community composition

Principal component analysis (PCA) was applied to the PLFA data, with principal components of 1 (PC1) and 2 (PC2) accounting for 75.6% of total variation in microbial community composition (Fig. 3). The PCA plot revealed that the soil microbial communities observed after different treatments were compositionally distinct. The variation in the PCA separated the combined treatments and PGPB treatments from control (Fig. 3A). Although the PLFAs of C5Ec and C5Ps were different, the PLFA of Ps and C5Ec were closely related. Notably, as indicated in the PCA of all treatment scenarios, the Gram-positive, Gram-negative and fungal communities were found to be higher in control samples as compared to treated samples, whereas PLFAs of eukaryotes were found to be strikingly higher in all treated samples except in the control treatment. The relative abundance of arbuscular mycorrhizal fungi, methanotrophs and actinomycetes were negatively correlated in control samples as compared to treated samples (Fig. 3B).

Microbial carbon biomass and soil respiration activities

A significant difference was observed in the MBC (Table 4) in the presence of ciliate and PGPB compared to control. The treatment with C5Ps increased the MBC to 223.59% (control) and 8.90% (Ps treatment) while C5Ec treatment increased the MBC to 310.57% (control) and 33.46% (Ec treatment). A similar activity was observed for the soil dehydrogenase enzyme. However, the alkaline phosphatase and FDA enzyme activities were greater in PGPB treated samples as compared to combined treatments. The alkaline phosphatase activity on different treatments was observed in the following sequence; $Ps > C5Ps > Ec > C5Ec > control$ whereas for FDA activity it was noted as $Ps > control > Ec > C5Ps > C5Ec$ (Table 4).

Nitrogen and phosphorus content of rice plant

The inoculation of ciliate-PGPB combination (C5Ps and C5Ec) moderately enhanced N and P uptake in the plant samples. The PGPB and ciliate treatments alone also showed a positive impact on total N and P (Table 5). Although this impact was much less compared to combined treatment, it was relatively significant compared to control plants.

Discussion

The interactions between a soil protist and PGPB have been demonstrated in the present study where *Enterobacter* sp. density negatively correlated with ciliate density, whereas *Pseudomonas* sp. proved to be resistant to predation under laboratory conditions. The results of this study are in agreement with

Pedersen [32] and Amacker [33], where *Pseudomonas* sp. was reportedly unaffected by ciliate predation. This may be due to the production of specific anti-predatory secondary metabolites by *Pseudomonas* sp. [22]. On the other hand, *Enterobacter* sp. are not known to produce toxic compounds. During pot culture studies, enhanced survival of both PGPB cultures was observed against predation. A possible explanation for this observation is the selective feeding of the introduced ciliate on competitors in the soil. This would have decreased the nutrient competition for *Enterobacter* sp. and hence aided in its survival [34].

Although a positive impact of rhizosphere protists (especially *Acanthamoeba* sp.) on plant productivity has been described repeatedly in literature; very few reports are available, at present, on the impact of protists on the survival of PGPB [5, 34]. For instance, the treatment of rice seedlings with amoebae developed a more ramified root structure and increased N uptake by the plants [23]. Similarly, our observations are in agreement with well-documented studies on the effect of soil protists on rice [23, 35] and other plants [5, 36, 37]. This observation may be due to the well-known microbial loop concept which explains the nutrient turn-over by protists preying on bacterial community and subsequently releasing bacterial biomass [38]. Therefore, the plants can take up considerable amount of readily available N due to the protist predation on combined treatments.

In our study, the highest increase in plant growth was obtained on combined treatment with *Kreyellidae* sp. and *Enterobacter* sp. It is known that the production of phytohormone by bacteria stimulates the plant growth [39] and recent reports have suggested that the predation by protist increases the IAA producing bacterial community in soil and thereby stimulates the early rice plant growth [34]. The current results of increased plant growth and higher densities of bacteria on combined treatment with *Kreyellidae* sp. and *Enterobacter* sp. further explains the positive impact of ciliate-PGPB interaction on plant growth performance. However, different protist and PGPB interaction should be studied to better understand the impact of their interaction on plant growth.

It has been reported in the previous studies that protist inoculation changes the soil microbial community composition [40, 41]. Our results are in agreement with these findings of top-down regulation by protists in structuring the soil microbiota. Different protist species are fundamentally differed in their grazing preferences [42]. For instance, inoculation of *Tetrahymena* increased the Gram-positive PLFAs, whereas, *Colpoda* led to an increase in Gram-negative PLFAs [15]. In our studies, *Kreyellidae* sp. decreased the PLFAs of Gram-positive as well as Gram-negative bacteria. The substantial decrease in Gram-positive bacterial community in combined and PGPB treatments was likely due to the competitive shifts in the bacterial community and preferential predation of ciliate [12, 43]. Our results suggested fundamentally different effects of ciliate grazer on overall soil microbial community in rice rhizosphere.

The MBC is an efficient parameter in evaluating the plant health since it involves determining the regulated nutrient cycles which is made available to plants [44]. In the present study, MBC was greater in microbial treated plants as compared to controls. This indicated that the ciliate-PGPB interaction could stimulate the microbial activity in soil. Similarly, the increased dehydrogenase activity on combined

treatment might be due to the presence of vast quantities of readily degradable organic substrates for microbial proliferation [44]. The difference in the alkaline phosphatase and FDA activities of the treated samples may be due to the presence of organophosphate complexes and alterations in microbial action during protist-PGPB interactions [45].

In conclusion, the ciliate-PGPB interaction provides a piece of evidence for enhanced plant growth at the lab as well as greenhouse scale studies. The PGPB and ciliate, together, significantly enhanced the plant growth through various mechanisms. These included altering the root structure of plants, increasing the uptake of N and P by plants, causing a shift in the bacterial communities and influencing the soil respiration enzymes. This is the first report describing ciliate (*Kreyellidae* sp.) interaction with two different plant growth promoting rhizobacterial species, and highlighting the effect of their interaction on plant growth, soil respiration and shifts in soil microbial communities.

Declarations

Ethical Approval

Not applicable

Consent to Participate

Not applicable

Consent to Publish

Not applicable

Authors Contributions

NA: designed the study and wrote the final manuscript; KAC and RSP: performed the experiment and analyzed the data; KAC: drafted the manuscript

Funding

Not applicable

Competing Interests

The authors declare that they have no conflict of interest

Availability of data and materials

All data generated or analyzed during this study are included in this published article

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Tables

Table 1. Impact of PGPB-ciliate interaction on plant growth in the plate assay

Treatment	Shoot length (cm)	Primary root length (cm)	# Seminal roots/plant	# Lateral roots/plant
Control	9.16±0.60 ^b	7.60±0.32 ^d	3.33±0.33 ^d	24.0±6.24 ^e
Ps	8.56±0.26 ^b	11.86±0.44 ^{ab}	4.67±0.66 ^c	41.0±12.00 ^d
C5Ps	9.53±0.48 ^{ab}	12.53±0.31 ^a	9.67±0.33 ^b	89.3±11.67 ^b
Ec	6.10±0.63 ^c	9.43±2.23 ^c	5.67±0.88 ^c	53.0±13.45 ^c
C5Ec	10.30±0.80 ^a	12.60±1.10 ^a	11.67±0.88 ^a	115.3±22.18 ^a

#- numbers; Data obtained are means of three replicates and analyzed using one-way analysis of variance (ANOVA). Mean values (± S.E.) in similar column with different lower-case are significantly

different (Tukey's HSD, $p < 0.05$). Ps-*Pseudomonas* sp; C5Ps- *Pseudomonas* sp and *Kreyellidae* sp; Ec- *Enterobacter* sp; C5Ec- *Enterobacter* sp and *Kreyellidae* sp.

Table 2. Plant growth promotion activity of PGPR-ciliate interaction in the pot experiment

Treatment	Shoot length (cm)	Root length (cm)	Shoot dry weight (g)	Root dry weight (g)
Control	19.6±1.13 ^d	8.3±0.47 ^e	0.10±0.08 ^e	0.05±0.06 ^c
Ps	30.5±1.33 ^c	14.9±1.48 ^d	0.18±0.08 ^d	0.09±0.08 ^{ab}
C5Ps	31.7±0.90 ^b	25.5±2.89 ^b	0.29±0.05 ^c	0.16±0.04 ^a
Ec	30.7±0.87 ^c	17.9±1.63 ^c	0.30±0.02 ^{ab}	0.08±0.06 ^{ab}
C5Ec	35.1±1.03 ^a	32.8±1.30 ^a	0.34±0.03 ^a	0.16±0.02 ^a

Data obtained are means of three replicates and analyzed using one-way analysis of variance (ANOVA). Mean values ($\pm$ S.E.) in similar column with different lower-case are significantly different (Tukey's HSD, $p < 0.05$). Ps-*Pseudomonas* sp; C5Ps- *Pseudomonas* sp and *Kreyellidae* sp; Ec- *Enterobacter* sp; C5Ec- *Enterobacter* sp and *Kreyellidae* sp.

Table 3. Enumeration of bacterial (CFU) and protists (MPN) population in the rhizosphere soils

Treatment	CFUs g ⁻¹	MPN g ⁻¹
Control	15.0±0.00 ^d ×10 ⁶	1.03±0.02 ^d
Ps	29.4±0.01 ^c ×10 ⁸	1.12±0.16 ^d
C5Ps	24.10±2.19 ^c ×10 ¹⁰	10.52±0.39 ^a
Ec	117.0±0.20 ^b ×10 ⁹	1.95±0.03 ^d
C5Ec	131.0±3.70 ^a ×10 ¹¹	5.12±0.06 ^c

Data obtained are means of three replicates and analyzed using one-way analysis of variance (ANOVA). Mean values ($\pm$ S.E.) in similar column with different lower-case are significantly different (Tukey's HSD, $p < 0.05$). Ps-*Pseudomonas* sp; C5Ps- *Pseudomonas* sp and *Kreyellidae* sp; Ec- *Enterobacter* sp; C5Ec- *Enterobacter* sp and *Kreyellidae* sp.

Table 4. Effect of PGPR-ciliate interaction on the MBC and soil respiration activity

Treatments	Microbial carbon biomass (mg C g ⁻¹ soil)	Alkaline phosphatase (μg p-NP g ⁻¹ soil h ⁻¹)	FDA analysis (μg fluorescein g ⁻¹ soil h ⁻¹)	Dehydrogenase (μg TPF g ⁻¹ soil h ⁻¹)
Control	36.88 ± 2.11 ^c	14.75±0.40 ^d	4.13±0.16 ^b	14.02±0.55 ^c
Ps	109.58 ± 4.67 ^b	28.39±0.37 ^a	5.46±0.23 ^a	14.60±0.31 ^c
C5Ps	119.34 ± 3.43 ^b	25.96±0.56 ^b	3.04±0.06 ^c	19.76±0.43 ^a
Ec	100.9 ± 2.84 ^b	25.06±0.73 ^b	4.08±0.04 ^b	16.78±0.52 ^b
C5Ec	151.42 ± 6.07 ^a	18.69±0.32 ^c	2.92±0.07 ^c	10.96±0.38 ^d

Data obtained are means of three replicates and analyzed using one-way analysis of variance (ANOVA). Mean values (± S.E.) in similar column with different lower-case are significantly different (Tukey's HSD, $p < 0.05$). Ps-*Pseudomonas* sp; C5Ps- *Pseudomonas* sp and *Kreyellidae* sp; Ec- *Enterobacter* sp; C5Ec- *Enterobacter* sp and *Kreyellidae* sp.

Table 5. Effect of PGPR-ciliate interaction on plant N and P uptake

Treatment	Nitrogen (%)	Phosphorous (%)
Control	1.66±0.22 ^b	0.173±0.012 ^{bc}
Ps	2.66±0.14 ^a	0.201±0.002 ^b
C5Ps	2.78±0.09 ^a	0.155±0.003 ^c
Ec	2.24±0.17 ^{ab}	0.108±0.002 ^d
C5Ec	2.76±0.11 ^a	0.273±0.015 ^a

Data obtained are means of three replicates and analyzed using one-way analysis of variance (ANOVA). Mean values (± S.E.) in similar column with different lower-case are significantly different (Tukey's HSD, $p < 0.05$). Ps-*Pseudomonas* sp; C5Ps- *Pseudomonas* sp and *Kreyellidae* sp; Ec- *Enterobacter* sp; C5Ec- *Enterobacter* sp and *Kreyellidae* sp.

Figures

Figure 1

Growth assessment assay of ciliate with PGPB (Ps and Ec) on solid and in liquid media. (A) Bacterial (Ec) growth elimination from the streaked line indicates feeding on bacteria by ciliate predator species (C5). Control- bacteria alone; C5 (+ve) - ciliate predation on bacteria; C4 (-ve) - negative control. (B) Graph indicates PGPB CFU count ml⁻¹ (log transformed) during the 5 days of incubation of PGPB with or without ciliate presence. (C) Graph indicates ciliate count in presence of PGPB during the 5 days of incubation in liquid media. Ec- *Enterobacter* sp; C5Ec- *Enterobacter* sp and *Kreyellidae* sp; Ps- *Pseudomonas* sp; C5Ps- *Pseudomonas* sp and *Kreyellidae* sp.

Figure 2

The effect of ciliate and PGPB interaction on 30 days old rice seedling growth. 1. Control (without any inoculation); 2. Ec- inoculation of *Enterobacter* sp; 3. C5Ec- inoculation of *Enterobacter* sp and *Kreyellidae* sp; 4. Ps- inoculation of *Pseudomonas* sp; 5. C5Ps- inoculation of *Pseudomonas* sp and *Kreyellidae* sp.

Figure 3

Principal component analysis (PCA) biplots based on (A) Microbial community composition in the soil samples of different treatments; (B) the relative abundance of different PLFAs. Ec- *Enterobacter* sp; C5Ec- *Enterobacter* sp and *Kreyellidae* sp; Ps- *Pseudomonas* sp; C5Ps- *Pseudomonas* sp and *Kreyellidae* sp.

Enterobacter sp and Kreyellidae sp; Ps- Pseudomonas sp; C5Ps- Pseudomonas sp and Kreyellidae sp.