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Cross-compatibility evaluation of plant growth promoting rhizobacteria of coconut and cocoa on yield and rhizosphere properties of vegetable crops

T.H. Khadeejath Rajeela, Murali Gopal*, Alka Gupta, Ravi Bhat, George V. Thomas

Microbiology Section,ICAR-Central Plantation Crops Research Institute, Kudlu P.O., Kasaragod, Kerala 671124, India

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ABSTRACT

Plant growth promoting rhizobacteria (PGPR), *Bacillus megaterium* TSB16 isolated from coconut and *Pseudomonas putida* KDSF23 from cocoa, were tested for cross-compatibility with vegetable crops in combination with coconut leaf vermicompost (CLV). The treatments included CLV @10 t/ha (T1), *B. megaterium* + CLV @ 6 kg /ha and 10 t/ha, respectively (T2), *P. putida* + CLV @ 6 kg /ha and 10 t/ha, respectively (T3) and recommended dose of NPK fertilizers @75:40:25 kg N, P_2O_5, K_2O + CLV @ 2.5 t/ha (T4). The results of the field trial indicated that the cumulative yield of tomato was significantly higher ($P < 0.05$) with chemical fertilizer application (278.7 g/plant) and *Pseudomonas putida* KDSF23+ CLV treatment (275.8 g/plant) compared to that in CLV application (239.5 g/plant). The yield of chilli was significantly higher in the plots that received chemical fertilizers. The soil N, P, K and organic carbon were highest in chemical fertilizer applied plots. However, a significant increase in population of rhizosphere microbial communities, particularly the plant-beneficial microbiota, and soil enzyme activities (phosphatase, dehydrogenase and urease) were recorded in the PGPR and CLV treated plots. The function-specific microorganisms *viz.* fluorescent pseudomonads, phosphate solubilizers, free-living nitrogen fixers and *Trichoderma* were 4.7–9.1, 3.5–3.8, 1.3–2.1 and 2.0–2.2 fold higher, respectively, in tomato and 1.5–1.6, 1.4–2.5, 1.3–1.4 and 2.9–3.4 fold higher, respectively, in chilli, in PGPR treated plots than that received chemical fertilizer. Our findings suggest that the PGPR strains isolated from the rhizosphere of coconut and cocoa can be utilized as a bioinoculant for vegetable production in organic agricultural systems indicating cross-compatible nature. They can also serve as a single bioinoculant for the main crop (coconut) and its intercrops (such as vegetables) in coconut based cropping system to reduce inorganic fertilizer application.

1. Introduction

On global scale, more than 175.7 million tonnes of chemical fertilizers are applied to soil annually (FAO, 2011) and have been found to be well correlated with the crop yield. However, they are one of the potent sources of environmental pollution that leads to resource degradation and negatively impact soil ecological functions (Kumar et al., 2014). Consequently, it is a great challenge to search for sustainable strategies to alleviate detrimental effects of intensive farming practices that consume chemical fertilizers. Effective biological technologies like the use of plant growth promoting rhizobacteria (PGPR) are being exploited for enhancing crop yield in modern agricultural systems in order to reduce the chemical fertilizer input. PGPR are soil- borne bacteria that have the ability to aggressively colonize the rhizosphere or plant roots stimulating the growth and yield of plants (Kloepper and Schroth, 1978). Studies have shown the positive effects of PGPR in increasing the growth of cereals (Lavakush et al., 2014), fruits (Kavino et al., 2010), vegetables (Gravel et al., 2007) and spices (Anandaraj and Sarma, 2003). Most of the research demonstrated the effectiveness of PGPR isolated from the rhizosphere of a particular crop on the yield and rhizosphere properties of the same crop. There are also reports of the effectiveness of PGPR isolated from one crop in improving the yield of other crops. Kandel et al. (2015) demonstrated that the diazotrophic endophytes of the eudicots poplar and willow colonized rice plants and enhanced plant growth by greater biomass, higher tiller number and taller plant stature than uninoculated control in N- limited conditions. Similarly, *Bacillus cereus,* isolated from annual crop, was capable of long-term colonization of cocoa foliage and reduced black pod rot disease severity caused by *Phytophthora capsici* in cocoa plants (Melnick et al., 2008). However, there is not much information available on cross-compatibility of PGPR isolated from plantation crops on annual or seasonal crops.

The coconut palm (*Cocos nucifera* L.) is a perennial tree crop grown

* Corresponding author.
E-mail address: mgcpcri@yahoo.co.in (M. Gopal).

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in over 90 countries in the tropics for diversified uses. Coconut, grown as a monocrop, is only marginally productive and profitable due to the poor utilization of resources in a coconut garden. The coconut farmers in India, particularly of Kerala State, are accustomed to grow a variety of annual and perennial crops in the interspaces of coconut palm to meet their various requirements. Cocoa, a plantation crop, is highly compatible and profitable mixed crop in coconut gardens. Vegetable crops are also grown predominantly as intercrops in coconut based homesteads of Kerala (Nayar, 2011). In our comprehensive study on agriculturally important microorganisms associated with coconut and cocoa, we isolated two plant growth promoting rhizobacteria, *Bacillus megaterium* TSB16 from rhizopshere of coconut and *Pseudomonas putida* KDSF23 from cocoa, with high potential for plant growth promotion (George, 2013; Thomas, 2013). It would be interesting to know if the same strains can be utilized as bioinoculants for the main as well as the intercrops. However, so far there have been no reports of PGPR isolated from plantation crops, particularly from coconut and cocoa, being used as bioinoculants for vegetable crops.

Therefore, a field experiment was conducted to study the effect of inoculation of PGPR, previously isolated from plantation crops, *viz.*, *Bacillus megaterium* TSB16 from the rhizosphere of coconut (George, 2013) and *Pseudomonas putida* KDSF23 from the rhizosphere of cocoa (Thomas, 2013), on yield of chilli and tomato cultivated as intercrops in coconut interspaces at ICAR-Central Plantation Crops Research Institute farm, (Kasaragod, Kerala, India) and its impact on nutrient status, microbial population and enzymes activities in rhizosphere soil.

2. Materials and methods

2.1. PGPR used in the study

Plant growth promoting rhizobacteria, *Bacillus megaterium* TSB16 and *Pseudomonas putida* KDSF23, used in this study were obtained from the glycerol stocks of these cultures maintained in the Microbiology Section, ICAR-CPCRI, Kasaragod, Kerala, India. These PGPR were originally isolated from the soils (5–15 cm depth) collected from the rhizosphere region of coconut palms and cocoa trees by standard procedures using the serial dilution technique. Ten-fold dilutions of soils collected from Kidu, Dakshina Kannada District, Karnataka State, India (Geographical location: 12°43′ N, 75°35′E; Cocoa var.: Criollo; Age: 12 years; Soil type: laterite; pH 5.3) were plated on King's B agar medium for isolation of *Pseudomonas putida* KDSF23 (Thomas, 2013). Standard heat treatment enrichment technique was used for obtaining *Bacillus megaterium* TSB16 (on nutrient agar medium) from coconut rhizosphere soil collected from Siddapura, Tumkur District, Karnataka State, India (Geographical location: 13°20′21″ N, 77°6′50″E; Coconut var.: Tiptur Tall; Age: 26 years; Soil type: sandy loam; pH 7.9) (George, 2013).

2.2. Preparation of talc-based formulations of PGPR

For the preparation of talc based biofertilizers of PGPR, *Bacillus megaterium* TSB16 and *Pseudomonas putida* KDSF23 were inoculated in to nutrient broth and King's B broth, respectively, and incubated at 33 °C for 48 h. Talc formulation of isolates were prepared by mixing the microbial culture with pre-sterilized talc powder under aseptic condition. The moisture content was maintained at 50% WHC, packed in polypropylene bag and sealed. The viable population of *Bacillus megaterium* TSB16 and *Pseudomonas putida* KDSF23 was 7×10^8 and 5×10^{10} cells per gram of carrier material, respectively.

2.3. Experimental site

Field experiment was conducted at the coconut farm (12″ 30″ N latitude and 75° 00′ E longitude with an altitude of 10.7 m above MSL) of ICAR-Central Plantation Crops Research Institute, Kasaragod, Kerala, India during December 2012 to March 2013. Mean maximum and minimum air temperature during the period were 31.2 and 23.6 °C, respectively, which is ideal for vegetable cultivation in this area. Average relative humidity during the cropping season was 61–94%. No rainfall was recorded in the growing season. The soil of the research site was sandy loam with pH 4.8, electrical conductivity of 15.1 μScm^{-1}, organic carbon content of 0.04%, and total nitrogen of 0.01%, 25.6 ppm available P and 17.4 ppm available K. The soil dehydrogenase, phosphatase and urease activities were 0.22 μg TPF/g soil/h, 17.8 μg PNP/ g soil/h and 27.4 μgNH_4/g soil/h, respectively. The population of bacteria, fungi, actinomycetes, *Trichoderma* species, fluorescent pseudomonads, phosphate solubilizers and free-living nitrogen fixers were found to be 40×10^5, 14×10^3, 16×10^4, 2×10^4, 7×10^2, 5×10^4 and 4×10^2 cfu (colony forming units)/g soil, respectively.

2.4. Experimental layout

The field experiment was conducted in an area of 81 m^2 within the interspaces of two rows of coconut palms (West Coast Tall variety, > 50 years age). The height of palms was more than 60 feet which allowed sufficient sunlight for cultivating intercrops. The 81 m^2 area was split into two plots of 40.5 m^2 each. The following four treatments were included for each vegetable crop:

1. T1 – Coconut leaf vermicompost (CLV) @10 t/ha
2. T2 -*Bacillus megaterium* @ 6 kg/ha + CLV @10 t/ha
3. T3 -*Pseudomonas putida* @6 kg/ha + CLV @10 t/ha
4. T4 –Recommended doses of NPK fertilizers @75:40:25 kg N, P_2O_5, K_2O + CLV @2.5 t/ha

The field experiment comprising the above four treatments was laid out in a randomized complete block design. Four replicates (one bed equal to one replicate) per treatment were maintained. The crops were irrigated with micro-sprinkler system ensuring proper moisture regime for the experimental plot. A basal application of coconut leaf vermicompost (CLV) produced in-house from coconut leaf substrate using local isolate of *Eudrilus* sp. at ICAR-CPCRI, Kasaragod, Kerala, India (Gopal et al., 2009) was applied @ 2.5 t/ha to the plot. Chemical fertilizers were applied at the recommended NPK dose (75 kg: 40 kg: 25 kg) for vegetables as per the package of practice of Kerala Agricultural University, Kerala, India. Talc formulation containing *Bacillus megaterium* and *Pseudomonas putida* was applied @ 6 kg/ha each, through soil inoculation.

2.5. Crop establishment and yield data

Healthy tomato and chilli seedlings raised on coir-pith compost in 100 cell seedling trays were obtained from Seed Production Centre, State Agriculture Department, Kasaragod, Kerala, India for the field studies. Tomato (var. Anagha) and chilli (var. Vellayani Athulya) seedlings were transplanted into the raised beds in their respective plots. A total of eighty tomato and ninety-six chilli seedlings were distributed equally within four treatments in the respective 40.5 m^2 plots. A replicate plot of tomato had five seedlings and chilli had six seedlings. Treatment application was initiated one week after the seedlings had established properly. For T1 treatment, CLV was added @ 10 t/ha in four split dose of 2.5 t/ha (about 1 kg per replicate plot). For T2 and T3 treatments, 3 g talc based PGPR formulation was mixed with 4 kg of soil and each bed received 1 kg of the PGPR-soil mix. The PGPR application was done twice during the field study, one week and one month after transplanting the seedlings. The CLV addition to T2 and T3 followed the same application method as in T1, the first two out of four splits coinciding with the PGPR application. The N, P and K in the chemical fertilizer treatment were supplied through the application of urea, rock phosphate and muriate of potash, respectively, after one week of planting the seedlings, in single dose as top dressing. The plots

were kept weed free by hand weeding. During the first month of the crops, two sprays of neem based insecticide were given to prevent insect attack. Once the crop entered the yielding stage, tomatoes and green chilli were harvested at regular intervals and their weights were immediately recorded. Finally, the cumulative yield was worked out by summation of the weights of each harvest.

2.6. Experimental methods

Rhizosphere soils were collected after the final harvest of tomato and chilli. From four replication beds per treatment, three beds were randomly selected. The plants were uprooted from each of the beds and rhizosphere soils were collected by shaking off the tightly adhering soils to the plant roots. Thus, twelve soil samples for each crop (24 for both crops) were obtained. The soils were collected in clean, labeled polythene bags and transferred to Microbiology laboratory where they were homogenized properly using the quadrant method. Each sample was split into two. One set was immediately stored at 4 °C for microbial and soil enzyme analyses and another set was air-dried and sieved for the nutrient analysis.

2.6.1. Soil chemical parameters

Soil pH was determined by suspending 10 g of soil in 25 ml distilled water using pH meter (Eutech Cyberscan, USA). Electrical conductivity was measured by mixing 10 g soil in 50 ml water using conductivity meter (Eutech, USA). The dried soil samples were analyzed for total organic carbon content as described by Walkley and Black (1934). Total nitrogen was estimated by Kjeldahl method (Kjeldahl, 1883). Available phosphorus was estimated spectrophotometrically at 660 nm using Bray P1 extract (Bray and Kurtz, 1945) and available potassium was determined by flame photometry using 1 N ammonium acetate (Hanway and Heidel, 1952).

2.6.2. Microbial population and soil enzyme analysis

The viable and culturable microbial counts were determined by standard serial dilution plate technique using selective medium for specific groups of microorganisms. Nutrient agar (Himedia, India), Ken Knights and Munaier's agar medium (Allen, 1959) and Martin Rose Bengal agar medium (Martin, 1950) were used for the enumeration of total bacteria, actinomycetes and fungi, respectively. Free-living nitrogen fixers were counted after 6 days incubation on N-free Jensen's agar medium (Becking, 1959). Colonies showing clear halo on Pikovskaya's agar medium (Pikovskaya, 1948) were counted as phosphate solubilizers. King's B agar (King et al., 1954) was used for fluorescent pseudomonads and the colonies fluorescing under UV light (360–400 nm) were enumerated after incubation for 24 h at 28 °C. *Trichoderma* specific medium was used to enumerate *Trichoderma* species after 7 days of incubation at 28 °C (Elad and Chet, 1983). The results of microbial enumeration were expressed as cfu (colony forming units)/g dry soil. The activities of soil enzymes *viz*. phosphatase (Tabatabai and Bremner, 1969), dehydrogenase (Klein et al., 1971) and urease (Tabatabai and Bremner, 1972) were also estimated.

2.7. Statistical analysis

The experiment was performed in a completely randomized block design with four replications per treatment. The experimental data were statistically analyzed by analysis of variance (ANOVA) using SAS software version 9.2 (Statistical Analysis System Institute, Cary, NC, USA). To determine the significance of difference between means of treatments, least significant difference (LSD) was computed at 5% probability level.

Table 1
Yield response in chilli and tomato upon inoculation of PGPR isolated from coconut and cocoa.

Treatment	Treatment detail	Chilli (g/plant)	Tomato (g/plant)
T1	CLV	14.5[b]	239.5[b]
T2	*Bacillus megaterium* TSB16 + CLV	18.1[b]	246.3[b]
T3	*Pseudomonas putida* KDSF23 + CLV	18.8[b]	275.8[a]
T4	Chemical fertilizer[*]	24.3[a]	278.7[a]

CLV- Coconut leaf vermicompost
Means followed by the same letter in a column do not differ significantly according to Fisher LSD test ($P < 0.05$).
[*] NPK@75 Kg N, 40 Kg P_2O_5, 25 Kg K_2O_5

3. Results

3.1. Effect of PGPR on yield of vegetables

The yield response of vegetables to the different treatments in the study is summarized in Table 1. Across all treatments, the application of chemical fertilizers resulted in higher yield ($P < 0.05$) in chilli (24.3 g/plant) and tomato (278.7 g/plant) compared to that in CLV treated plots. However, the application of the PGPR, *Pseudomonas putida* KDSF23, along with CLV resulted in significantly higher tomato yield (275.8 g/plant) that was on par with the yield under chemical fertilizer treatment. Higher yields of tomato and chilli were obtained in the PGPR+CLV applied plots compared to plots that received only CLV.

3.2. Effect of PGPR inoculation on soil chemical parameters

The results of the analysis of soils from rhizosphere of chilli and tomato are given in Tables 2 and 3. The pH of the rhizosphere soil was acidic in all plots which received different treatments and did not vary significantly among treatments. Electrical conductivity ranged from 37.4 to 48.9 μS cm^{-1} and 42.2–59.3 μS cm^{-1} in chilli and tomato, respectively, across the treatments. A substantial increase in pH and EC values along with enhanced N, P, K and organic carbon content was recorded in all the treatments compared with pre-experimental status of the soil. Within the treatments, N, P, K and organic carbon increase was highest in chemical fertilizer application followed by *P. putida* +CLV application for both the vegetable crops. The least change in N, P, K and organic carbon levels compared to pre-experimental status was recorded in plots that received only CLV..

3.3. Effect of PGPR on soil microbial status

Pre-treatment and post-treatment soil microbial status had a large difference in population of the microbial communities with the latter having almost double the counts in PGPR+CLV applied plots. Analysis of variance showed positive effect of PGPR+CLV on general microbial (bacteria, fungi, actinomycetes) and plant-beneficial communities (free-living nitrogen fixers, phosphate solubilizers, fluorescent pseudomonads, *Trichoderma* spp.) in rhizosphere soil of both the crops compared to chemical fertilizer application (Tables 4 and 5). Actinomycetes population did not show any significant difference across various treatments in chilli soils, whereas, in tomato, the PGPR+CLV plot had significantly high population of this microbial community. Counts of fluorescent pseudomonads were found to be high in tomato (31.9×10^2 cfu/g dry soil) and chilli (14.4×10^2 cfu/g dry soil) plots applied with *P. putida* KDSF23. *Trichoderma*, an important antagonist, was also found in higher numbers in the soils of both crops when applied with PGPR+CLV.

Table 2
Impact of coconut and cocoa PGPR on physico-chemical and nutrient properties in the rhizosphere soil of chilli plants.

Treatment	Treatment detail	pH	Electrical conductivity (µS/ cm)	Organic carbon (%)	Total nitrogen (%)	Available phosphorus (ppm)	Available potassium (ppm)
T1	CLV	5.3	48.90	0.38^{c}	0.03^{c}	50.1^{c}	46.5^{c}
T2	*Bacillus megaterium* TSB16 + CLV	5.2	47.7	0.39^{c}	0.05^{b}	61.6^{b}	76.3^{b}
T3	*Pseudomonas putida* KDSF23+ CLV	5.2	37.4	0.53^{b}	0.06^{b}	55.6^{bc}	89.0^{b}
T4	Chemical fertilizer*	5.4	41.9	0.59^{a}	0.08^{a}	81.4^{a}	115.7^{a}

CLV- Coconut leaf vermicompost
Means followed by the same letter in a column do not differ significantly according to Fisher LSD test ($P < 0.05$).
* NPK@75 Kg N, 40 Kg P_2O_5, 25 Kg K_2O.

3.4. Effect on soil enzymes

The data on three soil enzymes, dehydrogenase, phosphatase and urease, in the rhizosphere of chilli and tomato are presented in Tables 6 and 7. PGPR+CLV application positively influenced the soil enzyme activities when compared to other treatments. Soil application of *Pseudomonas putida* KDSF23+CLV enhanced ($P < 0.05$) the activities of phosphatase and urease in the rhizosphere soil of both the vegetable crops. In addition, dehydrogenase activity in rhizosphere soil of tomato (1.30 µg TPF/ g soil/h) was significantly enhanced when inoculated with *Bacillus megaterium* TSB16+CLV. Except urease, the other two enzymes recorded least activity in the chemical fertilizer applied plots of both crops.

4. Discussion

The study indicated that the application of *Pseudomonas putida* KDSF23+CLV resulted in higher tomato yield, which was at par with chemical fertilizer treatment. This meant that the PGPR could sustain the yield levels equivalent to those obtained using recommended NPK dose, when applied along with suitable organic amendment such as vermicompost (CLV). However, in case of chilli, the treatment under recommended NPK dose recorded significantly higher yield than the application of PGPR +CLV and only CLV. This might be due to the higher N, P and K contents of rhizosphere soil treated with chemical fertilizer. Between the two PGPR, it was observed that *P. putida* was able to elicit better yield response when compared to *B. megaterium*. Previous findings of Manikandan et al., (2010) revealed that the inoculation of *Pseudomonas fluorescens* increased fruit yield of tomato compared to untreated control under glass house and field conditions. We are not sure whether the reasons for better yield in our studies were due to the better response of solanaceae crops, particularly tomato, to gram negative bacterium (pseudomonad), as was earlier reported (Gravel et al., 2007; Manikandan et al., 2010) or because of the higher population of *P. putida* (5×10^{10} cfu/g talc) in the bioformulation compared to *B. megaterium* (7×10^{8} cfu/g carrier) that we used for the field application.

We also noticed that there was synergistic impact with application of PGPR and CLV compared to sole application of CLV. It could be explained by the growth promotion mechanism of PGPR along with the favourable effect of CLV on chemical, physical and biological properties of soil. Coconut leaf vermicompost (CLV) acts as a good organic amendement as its addition improves the organic carbon, major and minor nutrients, plant growth promoting hormones such as cytokinins and gibberellins and humic acid content in the soil (Gopal et al., 2010). In addition to these, CLV, biologically, is powerhouse of plant-beneficial microbial communities (Gopal et al., 2009) that positively impact the soil health by increasing the microbial biomass and altering the microbial community structure (Gopal et al., 2012). Similar synergistic effects of growth promotion of pearl millet were reported by Hameeda et al. (2006) on inoculation of PGPR along with glyricidia vermicompost. Application of vermicompost was found to increase tomato yield, quality and soil fertility compared to chicken compost, horse compost and chemical fertilizer in greenhouse condition under different soil water regimes (Yang et al., 2015). The combination effect of compost+PGPR not only improved the crop yield, but also helped in reducing chemical fertilizer inputs to a great extent. Fifty percent of the recommended N could be saved in maize on application of vegetable waste compost with *P. fluorescens* in integrated nutrient management trials (Ahmad et al., 2008).

Of the two PGPR strains tested, effect of *Pseudomonas putida* on vegetable yield was better than that of *Bacillus megaterium*. A plausible explanation could be its potential to produce siderophores, indole acetic acid and ACC deaminase. Previous studies proved that the strain had significantly increased the total seedling length and total dry weight of cocoa plants in poly bags (Thomas, 2013). The IAA producing trait of the *Pseudomonas putida* might have stimulated the proliferation of the plant root system which led to the efficient uptake of nutrients from the soil that aided plant growth. Presence of genes responsible for providing the plant-beneficial properties was confirmed by whole genome analysis using next-generation sequencing platform (Gupta et al., 2014). IAA production by PGPR also enhanced root length in coconut seedlings which led to better growth and development of plants (George et al., 2013). It could be surmised that PGPR

Table 3
Impact of coconut and cocoa PGPR on physicochemical and nutrient properties in the rhizosphere soil of tomato plants.

Treatment	Treatment detail	pH	Electrical conductivity (µS/ cm)	Organic carbon (%)	Total nitrogen (%)	Available phosphorus (ppm)	Available potassium (ppm)
T1	CLV	5.6	42.2	0.40^{b}	0.03^{c}	42.0^{d}	38.8^{c}
T2	*Bacillus megaterium* TSB16 + CLV	5.4	54.7	0.41^{ab}	0.05^{b}	55.6^{b}	65.7^{b}
T3	*Pseudomonas putida* KDSF23 + CLV	5.5	59.3	0.50^{a}	0.05^{b}	46.8^{c}	83.1^{b}
T4	Chemical fertilizer*	5.4	56.0	0.52^{a}	0.08^{a}	92.9^{a}	119.9^{a}

CLV- Coconut leaf vermicompost
Means followed by the same letter in a column do not differ significantly according to Fisher LSD test ($P < 0.05$).
* NPK@75 kg N, 40 kg P_2O_5, 25 kg K_2O

Table 4
Effect of PGPR isolated from coconut and cocoa on plant-beneficial microbial status in rhizosphere soil of chilli plants.

Treatment	Treatment detail	Microbial population (cfu/g dry soil)						
		Bacteria ($x10^5$)	Fungi ($x10^3$)	Actino mycetes ($x10^4$)	*Trichoderma* ($x10^4$)	Fluorescent pseudomonads ($x10^2$)	Phosphate solubilizers ($x10^4$)	Free-living nitrogen fixers ($x10^2$)
T1	CLV	148.1^{b}	51.3^{c}	28.7^{a}	2.8^{b}	3.3^{c}	2.3^{c}	36.7^{b}
T2	*Bacillus megaterium* TSB16 + CLV	159.4^{ab}	84.7^{a}	31.8^{a}	7.3^{a}	14.9^{a}	28.9^{a}	51.8^{a}
T3	*Pseudomonas putida* KDSF23 + CLV	176.4^{a}	74.3^{ab}	21.4^{a}	8.6^{a}	14.4^{ab}	15.9^{b}	53.8^{a}
T4	Chemical fertilizer*	94.2^{c}	65.8^{b}	23.6^{a}	2.5^{b}	9.6^{b}	11.7^{b}	39.6^{b}

CLV- Coconut leaf vermicompost
Means followed by the same letter in a column do not differ significantly according to Fisher LSD test ($P < 0.05$).
* NPK@75 kg N, 40 kg P_2O_5, 25 kg K_2O

inoculation combined with CLV application provided considerable yield benefits to vegetable crops even though the strains were originally isolated from the rhizosphere of plantation crops. It is, thus, suggested that a single bioinoculant could be utilized for coconut gardens intercropped with vegetables, as vegetables are cultivated predominantly as an intercrop in coconut gardens (Nayar, 2011).

Application of PGPR+CLV improved the soil nutrient status in comparison to application of CLV alone, but not when compared to chemical fertilizer treatment. On content basis, inorganic chemical fertilizers have very high percentage of nitrogen, phosphate and potash when compared to organic inputs such as compost/vermicompost. The chemical fertilizer treatment had higher organic carbon too due to the basal application of coconut leaf vermicompost. Thus, it is expected that the chemical fertilizer treated plots would give maximum yield, but continuous use of chemical fertilizers for enhanced crop productivity often results in loss of soil health, unexpected harmful environmental effects and eutrophication of aquatic ecosystems (Adesemoye et al., 2009). Plant growth promoting rhizobacteria could reduce the use of chemical fertilizers to large extent (Minaxi and Saxena, 2011) as they possessed multifarious growth promoting traits (George et al., 2013) that contributed towards promotion of plant growth and yield.

Though the contribution of PGPR and compost might not have added nutrients to the soil to the extent chemical fertilizers did, the combination is reported to improve the use efficiency of the plant nutrients present in the soil, which is key to better crop production. Application of PGPR with P- enriched compost to the chick pea under irrigated rainfed soil increased N, P and K content of soil (Shahzad et al., 2014). Synergy between vermicompost and PGPR agents improved soil quality and crop yield of tomato and spinach (Song et al., 2015). The use of *Bacillus* species in different crops stimulated macro- and micro-nutrient uptake such as N, P, K, Ca, Mg, Fe, Mn, Zn and Cu (Elkoca et al., 2008; Gunes et al., 2009). It is likely that the increase in soil available P might have resulted due to increased population of phosphate solubilizing microorganisms and phosphatase activity which have an important role in mineralizing P present in soil, especially in high P-fixing acidic soils of Kerala (Sureshkumar et al., 2013). Phosphate solubilizing microorganisms produce a variety of organic acids including gluconic, oxalic, malic, and succinic acid, which solubilize inorganic phosphate to plant available forms (El-Tarabily et al., 2008). As a consequence of higher microbial densities and enzymatic activity, C, P and N turnover is increased in the rhizosphere (Castro-Sowinski et al., 2007). *Bacillus megaterium* TSB16 showed ammonification property, which is an important step in the transformation of organic nitrogen to ammoniacal form, that would have increased the availability of nitrogen to the plant. Seed vectored rhizobacteria of grasses such as *Festuca arundinaceae* Schreb, *Lolium perenne* L. and *Poa annua* L. had enhanced acquisition of organic-N from soils confirmed through ^{15}N isotopic studies (White et al., 2015). Direct N-fixation by diazotrophic *Bacillus sphaericus* UPMB-10 was found responsible for supply of up to 63% of nitrogen requirement of young immature oil palm using ^{15}N isotopic method (Zakry et al., 2012). It would be easy to assume that similar mode of action would have taken place in our studies too for enhanced nitrogen acquisitions by tomato and chilli that must have improved the nitrogen use efficiency.

Changes in the number of beneficial microorganisms in soil, their activities and alterations in their microbial biomass are indicators of potential and actual soil fertility (Schloter et al., 2003). Positive changes in soil microbial properties were more evident in the treatments that had biological inputs than the chemicals in the field studies we conducted to evaluate the efficacy of PGPR. The soil application of PGPR+CLV significantly enhanced the soil microflora especially the phosphate solubilizers, free-living nitrogen fixers and *Trichoderma* spp. Increased population of free-living nitrogen fixers and phosphate

Table 5
Effect of PGPR isolated from coconut and cocoa on plant-beneficial microbial status in rhizosphere soil of tomato plants.

Treatment	Treatment detail	Microbial population (cfu/g dry soil)						
		Bacteria ($x10^5$)	Fungi ($x10^3$)	Actino mycetes ($x10^4$)	*Trichoderma* ($x10^4$)	Fluorescent pseudomonads ($x10^2$)	Phosphate solubilizers ($x10^4$)	Free-living nitrogen fixers ($x10^2$)
T1	CLV	105.3^{b}	27.8^{c}	16.4^{c}	5.1^{a}	12.5^{b}	17.0^{b}	68.6^{bc}
T2	*Bacillus megaterium* TSB16 + CLV	151.7^{a}	54.8^{a}	36.8^{ab}	4.8^{a}	16.4^{b}	28.7^{a}	76.5^{b}
T3	*Pseudomonas putida* KDSF23 + CLV	148.5^{a}	44.2^{b}	39.0^{a}	5.1^{a}	31.9^{a}	31.9^{a}	128.6^{a}
T4	Chemical fertilizer*	82.4^{c}	40.5^{b}	28.0^{b}	2.4^{b}	3.5^{c}	8.3^{c}	58.6^{c}

CLV- Coconut leaf vermicompost
Means followed by the same letter in a column do not differ significantly according to Fisher LSD test ($P < 0.05$).
* NPK@75 kg N, 40 kg P_2O_5, 25 kg K_2O

Table 6
Effect of coconut and cocoa PGPR on enzyme activities in rhizosphere soil of chilli plants.

Treatment	Treatment detail	Phosphatase (μgPNP/g soil/h)	dehydrogenase (μgTPF/g soil/h)	Urease (μgNH_4/g soil/h)
T1	CLV	33.8[a]	0.88[ab]	36.9[d]
T2	*Bacillus megaterium* TSB16 + CLV	34.8[a]	1.13[a]	118.3[b]
T3	*Pseudomonas putida* KDSF23 + CLV	36.5[a]	1.14[a]	133.8[a]
T4	Chemical fertilizer*	29.0[b]	0.61[b]	58.3[c]

CLV- Coconut leaf vermicompost
Means followed by the same letter in a column do not differ significantly according to Fisher LSD test ($P < 0.05$).
* NPK@75 kg N, 40 kg P_2O_5, 25 kg K_2O.

Table 7
Effect of coconut and cocoa PGPR on enzyme activities in rhizosphere soil of tomato plants.

Treatment	Treatment detail	Phosphatase (μg PNP/g soil/h)	dehydrogenase (μg TPF/g soil/h)	Urease (μg NH_4/g soil/h)
T1	CLV	36.2[b]	0.68[b]	41.9[c]
T2	*Bacillus megaterium* TSB16 + CLV	37.7[ab]	1.30[a]	84.5[b]
T3	*Pseudomonas putida* KDSF23 + CLV	40.2[a]	1.06[a]	104.0[a]
T4	Chemical fertilizer*	26.0[b]	0.19[c]	53.3[c]

CLV- Coconut leaf vermicompost
Means followed by the same letter in a column do not differ significantly according to Fisher LSD test ($P < 0.05$).
* NPK@75 kg N, 40 kg P_2O_5, 25 kg K_2O.

solubilizers would have increased the supply of N and P nutrients to plant, thereby, improving their growth and yield. Our observation is supported by Cakmakci et al. (2007) who found that inoculation of *Bacillus megaterium* significantly increased the population of P-solubilizers in the rhizosphere of barley plants, thereby, promoting the crop growth to greater extent than uninoculated plants.

Trichoderma is one of most ubiquitous antagonist present in soil with broad spectrum pathogen suppressing and plant growth promoting properties (Lugtenberg et al., 2013). In our studies, application of PGPR+CLV or CLV alone enhanced the population of *Trichoderma* in rhizosphere of both the crops. This would have resulted in the suppression of common fungal pathogens such as *Fusarium*, *Rhizoctonia* and *Pythium* present in the soil capable of causing disease in vegetable crops. Now, it is emerging that using rhizosphere microbiome with composts is a futuristic technology for managing plant pathogens (Gopal et al., 2013).

Compared to biological inputs, the chemical fertilizer application was found to cause a significant drop in microbial communities. Imbalanced use of fertilizers hadbeen found to negatively impact the microbial count (Singh et al., 2015). The positive consequence of PGPR inoculation on microbial population might have increased the delivery of essential nutrients, thereby, altering the growth rate and metabolic activities of the crop, which in turn, might have resulted in more root exudates and created a favourable habitat for the growth and development of microorganisms in general.

PGPR application along with CLV also enhanced soil enzyme activities in rhizosphere soil. Increased soil enzyme activity seemed to rely on the increase in microbial population as a consequence of the inoculation treatment. The measurement of soil enzyme dehydrogenase is a direct indicator of soil microbiological activities, as there exists a direct co-relation between them. In our study, dehydrogenase activity in PGPR treated plots was greater compared to that in chemical treatment. It was observed that the soil having highest dehydrogenase activity also had greater population of microbial communities such as bacteria, fungi and actinomycetes. Our findings corroborated those of Aseri and Tarafdar (2006) who reported that increased soil enzyme activities might be related to an increase in microbial population. Soil inoculation with biofertilizers had been found to enhance the activities of dehydrogenase and alkaline phosphatase in rhizosphere soils of pomegranate compared to that of uninoculated control plants (Aseri et al., 2008). Also, measurement of enzymatic activities could provide an early indication of changes in soil fertility, since they are involved in mineralization of essential nutrient elements (Ceccanti et al., 1994). The activity of soil enzymes was less in chemical fertilizer treatment than that of PGPR inoculation. Exclusive use of chemical fertilizers had been found to cause a significant reduction in soil dehydrogenase and phosphatase activity in soils under rainfed ginger (Dinesh et al., 2012). Thus, the overall response of the biological properties (microbial and enzyme) in this study highlights the unfavorable impact of chemical fertilizers on soil even though it was applied along with CLV @2.5 t/ha.

In conclusion, the PGPR *Bacillus megaterium* TSB16 (isolated from coconut rhizosphere) and *Pseudomonas putida* KDSF23 (isolated from cocoa rhizosphere), proven for their ability to promote growth of coconut and cocoa seedlings, respectively, were found to be effective bioinoculants for vegetable crops such as tomato and chilli, when grown as intercrops in coconut garden. The use of PGPR with multifarious plant growth promotion properties resulted in an increase in the microbial population in the rhizosphere and improved production characteristics of soil. Though chemical fertilizer application gave highest yield in both the vegetable crops, *Pseudomonas putida* KDSF23+coconut leaf vermicompost addition resulted in tomato yield on par with the recommended dose of chemical fertilizer. The PGPR +CLV application produced a positive rhizosphere microbial feed-back in terms of significant improvement in general and function-specific plant-beneficial microbial population in rhizosphere of both the crops. The soil enzyme activities *viz.* dehydrogenase, phosphatase and urease were also recorded to be high in the PGPR+CLV applied plots which indicated a high exocellular enzyme production responsible for gaining access to nutrients bound to soil organic matter. Results of our study showed for the first time that PGPR from plantation crops, such as coconut and cocoa, are cross-compatible with vegetable crops and, thus, can be used as a bioinoculant for both the main and the component crops in a coconut- based cropping system.

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