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ORIGINAL PAPER

# Multifarious beneficial traits and plant growth promoting potential of *Serratia marcescens* KiSII and *Enterobacter* sp. RNF 267 isolated from the rhizosphere of coconut palms (*Cocos nucifera* L.)

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**Abstract** Two plant growth promoting bacteria designated as KiSII and RNF 267 isolated from the rhizosphere of coconut palms were identified as *Serratia marcescens* and *Enterobacter* sp. based on their phenotypic features, BIOLOG studies and 16S rRNA gene sequence analysis. Both bacteria exhibited phosphate solubilization, ammonification, and production of indole acetic acid, $\beta$-1, 3 glucanase activities and 1-aminocyclopropane-1-carboxylate-deaminase activity. They could also tolerate a range of pH conditions, low temperature and salinity (NaCl). In addition, *S. marcescens* KiSII exhibited N- fixation potential, chitinase activity, siderophore production and antibiotics production. Seed bacterization with these bacteria increased the growth parameters of test plants such as paddy and cowpea over uninoculated control in green house assay. In coconut seedlings, significant increase in growth and nutrient uptake accompanied with higher populations of plant beneficial microorganisms in their rhizospheres were recorded on inoculation with both the PGPRs. The present study clearly revealed that PGPRs can aid in production of healthy and vigorous seedlings of coconut palm which are hardy perennial crops. They offer a scope to be developed into novel PGPR based bioinoculants for production of elite seedlings that can benefit the coconut farming community and the coconut based ecology.

**Keywords** *Serratia marcescens* · *Enterobacter* sp. · Plant growth promotion · Perennial crop · Coconut seedlings

## Introduction

Coconut is an important plantation crop grown in more than 90 countries globally. India is the third largest coconut producing country and contributes about 16.51 percent in area and 26.65 percent in production of coconut in the world. Annual production is about 10,824 million nuts with an average of 5,711 nuts per hectare as per 2009–2010 statistics (http://agricoop.nic.in/Agristatistics.htm). Coconut has been eulogized in India as 'Kalpavriksha' meaning "Tree of Life" due to its multiple uses. Being a perennial crop, coconut palm mines nutrients from soil constantly. Application of fertilizers, therefore, becomes very important to restore the soil fertility. Inclusion of organic inputs such as compost, green manures and biofertilizers form an important method for improving the soil health and fertility and sustain crop production in coconut. One of the current areas of research in the field of biofertilizer technology includes the exploitation of plant growth promoting rhizobacteria (PGPR) for improving plant growth promotion, soil nutrient management and biological control (Vessey 2003). PGPR inoculants can fulfill diverse beneficial interactions in plants leading to promising solutions for sustainable and environment-friendly agriculture (Bhattacharyya and Jha 2012).

Several reports have shown that PGPR application causes significant increase in growth and yield of economically important crops (Shoebitz et al. 2009; Mehnaz and Lazarovits 2006) that include strains of *Enterobacter* sp. and *Serratia* sp. too (Taghavi et al. 2010; Chakraborty

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et al. 2010; Shoebitz et al. 2009). The exploitation of efficient PGPRs from the rhizosphere of perennial crop like coconut assumes particular significance as coconut palms are anchored to soil for many decades. In this study, two native strains were isolated from the rhizosphere of coconut; characterized and evaluated for their plant growth promotion capabilities under green house and field conditions. This is, to our knowledge, the first article that reports the plant growth promoting potential of *Serratia* sp. and *Enterobacter* sp. associated with coconut.

## Materials and methods

### Isolation

The PGPRs were isolated from the rhizospheric soil samples of coconut palms collected from Kidu, Karnataka, India (geographical location: 12°43′N, 75°35′E; soil type: laterite; pH 5.2; coconut variety: West Coast Tall (WCT)) and Ratnagiri, Maharashtra, India (geographical location: 16°52′ N, 73°18′E; soil type: sandy loam; pH: 5.8; coconut variety: Chowghat Orange Dwarf (COD)). Soil dilutions were plated on King's B agar (KBA) and isolates were purified on KBA plates. The isolated colonies were then streaked on KBA slants and stored at 4 °C and stock culture was maintained at −23 °C in nutrient broth (NB) supplemented with 15 % glycerol.

### Identification and characterization of the isolates

#### *Identification by conventional methods*

PGPRs were tentatively identified by standard morphological, physiological and biochemical methods (Cappuccino and Sherman 1992) according to Bergey's Manual of Determinative Bacteriology (9th edition).

#### *Identification of isolates by Biolog carbon substrate utilization patterns*

Identification of the isolates was validated and metabolic profile was generated by using Gen-III microplates of the Biolog system (Biolog, CA, USA). Cells grown for 24 h on Biolog universal growth (BUG) agar were collected and processed according to the manufacturer's instructions (http://www.biolog.com). Briefly, cultures were transferred to Inoculating fluid A (IF A) and inoculum density was adjusted to 98 % T using Biolog turbidimeter. Using multi channel pipette, cell suspension was inoculated into Biolog Gen III Microplates (100 μl/well) containing 96 wells that provides 94 phenotypic tests. Plates were incubated at 33 °C for 24 h. The optical density at 590 nm produced from the reduction of tetrazolium violet in each well was read after 24 h using a Biolog Microplate reader (version 5.1.1). Identification was attained when it compared the pattern formed in the well with possible patterns in the database (Microstation/Microlog Version 5.1.1). A species identification acknowledged when the similarity index (SIM) and distance (DIS) values were >0.500 and <5.00, respectively.

#### *Identification by molecular method*

Genomic DNA of the PGPRs was isolated by GenElute Bacterial Genomic DNA Kit (Sigma, USA). Fragment of 16S rDNA gene was amplified by PCR from the isolated genomic DNA using the universal primers F27/R1492. Amplification reactions contained 10X PCR buffer (2.5 μl), 2.5 mM dNTPs (2 μl), 25 mM $MgCl_2$ (1.5 μl), 5U/μl Taq DNA polymerase (0.125 μl) and 20 μM primers F27 and R1492 (1.25 μl each). The amplifications were performed using Veriti Thermal cycler (Applied Biosystems). The reaction conditions included an initial denaturation of 3 min at 94 °C followed by 30 cycles of 1 min at 94 °C, 30 s at 55 °C and 45 s at 72 °C with a final extension of 10 min at 72 °C and then the samples were cooled to 4 °C. Amplified products were resolved by electrophoresis in 0.8 % agarose gel and the expected band of 1.5 kb was observed. PCR amplicons were purified using the PCR purification kit (Zymo Research, USA) to remove contaminants. Purified DNA was subjected to capillary sequencing (3730 xl 96 Capillary Analyzer, Applied Biosystems). Forward and reverse DNA sequencing reaction of PCR amplicon was carried out using 27F and 1492R primers using BDT v3.1 cycle sequencing kit. The 16S rRNA gene sequences were analyzed by alignment with the GenBank database using BLAST (NCBI BLAST$^R$ home page). The nucleotide sequences of 16S rDNA segment determined in this study were deposited in NCBI GenBank database.

#### *Stress tolerance studies*

The tolerance of the plant growth promoting isolates towards abiotic stresses like high/low pH, high/low temperature and high salinity was studied. 100 μl of cultures (with population count of $10^8$ c.f.u. $ml^{-1}$) were added to trypticase soya broth (TSB) prepared using citrate buffer, phosphate buffer and Tris–HCl buffer for maintaining pH of 4.2, 5.2, and 6.2, 7.2 and 8.2, 9.0 respectively. After 3 days incubation, 1 ml of the culture was centrifuged at 15,000 rpm at 4 °C for 15 min. Pellet was suspended in 3 ml of the sterile distilled water and OD was read at 600 nm to determine the pH tolerance. Tolerance to temperature was studied by streaking the isolates on TSA plates and incubated at different temperatures viz. 4, 15, 30, 40, 50, 55 and 60 °C. Salt tolerance was detected on TSA plates amended with NaCl at different concentrations, 2, 4, 6, 8, 10 %, and incubated at 30 °C. Growth observations were recorded up to 7 days.

## Assays for plant growth promotion traits

### *Production of indole acetic acid (IAA)*

The cultures (500 µl of $1 \times 10^8$ c.f.u. $ml^{-1}$) were grown in 50 ml Luria–Bertani (LB) broth amended with L-tryptophan (5 mM $l^{-1}$) for a period of one week in dark condition at 30 °C at 180 rpm in a refrigerated incubator shaker (Innova Model 4335; USA). Extraction of IAA was done with equal volume of pre-cooled diethyl ether in a separatory funnel at 4 °C for 2 h with intermittent shaking. The process was repeated twice with 50 ml of diethyl ether and IAA present in the ether phase was collected and evaporated to dryness in a rotary vacuum evaporator (Equitron, India). To the dried material, 2 ml of methanol was added and kept at −22 °C until analysis. IAA present in the methanol extract was estimated by the standard procedure using Salkowski reagent (Gordon and Weber 1951).

### *Phosphate solubilization*

Bacterial suspension having $2 \times 10^7$ c.f.u. $ml^{-1}$ of cells was inoculated into Pikovskaya's broth containing insoluble tri-calcium phosphate (0.5 %) and incubated for a period varying from 24, 48, 72, 96, 120 and 144 h at 30 °C. Water soluble phosphorus in the culture supernatant was estimated by the chlorostannous reduced molybdophosphoric acid blue method as described by Jackson (1967).

### *Production of lytic enzymes*

Chitinase and $\beta$-1,3-glucanase activity were estimated as described by Lim et al. (1991). Reducing sugar equivalents produced in the solutions was measured by the spectrophotometric (Shimadzu UV 1601) method of Miller (1959) at 540 nm. Protein content in the samples was determined by the method of Bradford (1976) using bovine serine albumin as the standard.

### *Production of ACC-deaminase activity, siderophores, antibiotics, ammonia and HCN*

Qualitative detection of ACC-deaminase activity (Klee et al. 1991), siderophores (Schwyn and Nielands 1987), growth on N-free medium (on Jensen's medium), production of antibiotics (agar well technique by Fuhrmann 1994), volatile compounds like ammonia (Cappuccino and Sherman 1992) and HCN (Bakker and Schipper's 1987) were also done.

## Green house evaluation of isolates on short duration crops

Both the bacterial isolates were evaluated for their plant growth promoting potential on paddy and cowpea seedlings in green house. Surface sterilized paddy (Variety *Aiswarya*) and cowpea seeds (Variety *Kairali*) were sown in plastic cups (four seeds/cup) filled with unsterile soil: sand mixture in 3:1 ratio. One ml of 24 h old culture broth was applied on each seed. After germination, seedlings were thinned to 2 numbers per cup. Ten replications were maintained for each culture. Control was maintained by placing sterile seeds with out culture. The plants were irrigated at two day intervals. Observations were taken on 20th day of sowing. Shoot and root lengths, fresh and dry weight were recorded. Shoot length, root length and total seedling length were statistically analyzed.

## Poly bag assay on coconut seedlings

Bacterial isolates were tested for their plant growth promoting effect on coconut seedlings in polybags under field conditions. Coconut sprouts of West Coast Tall (WCT) variety were obtained from the farm of Central Plantation Crops Research Institute (Kerala, India). Each of the bacterial isolate were inoculated in 20 L King's B broth and incubated for 48–60 h so as to reach $10^8$ cells $ml^{-1}$. This culture broth was used as inoculum for coconut seedlings. Root portion of the sprouts were dipped in culture broth for 12 h and were transplanted to polybags containing soil, vermicompost and sand in the ratio 3:1:1 (@ 25 kg polybag $^{-1}$. One litre of culture broth was added to each polybag. Seedlings without bacterial treatment served as control. Twenty replications of coconut seedlings were maintained for each treatment in a completely randomized design. All the routine cultural operations like weeding and irrigation (once in 5 days) were carried out. One litre of respective bacterial culture ($10^8$ cells $ml^{-1}$) was applied to each polybag as a booster dose after 3 months of planting. After 6 months of planting, the seedlings were uprooted and growth parameters like length (LL), width (LW) and number (L No.) of leaves, length of root (RL) and shoot (SL), collar girth of seedling (GS), number of roots (R No.), fresh weight of shoot (FWS) and root (FWR), total fresh weight of seedling (TFW), dry weight of shoot (DWS) and root (DWR) and total dry weight of seedling (TDW) were determined. The oven dried samples were ground for estimation of phosphorus (P) and potassium (K) (Jackson 1967). Total nitrogen in the samples was estimated using N analyser (Gerhardt Vapodest, UK) following the Kjeldahl method. After estimating the nutrient concentration of the seedling (above ground portions), the nutrient uptake value for NPK was calculated on the basis of dry matter. The c.f.u. of beneficial microbes like N-fixers, phosphate solubilizers, fluorescent pseudomonads and *Bacillus* spp. in the rhizopshere of coconut seedlings were enumerated. Data were log transformed and expressed as $\log_{10}$ c.f.u. $\mathrm{ml}^{-1}$ before subjecting to ANOVA.

### *Statistical analysis*

The data were analysed by using SPSS statistical software (Ver. 15) and one way analysis of variance was done with ANOVA procedure in SPSS. And comparison among treatments was done by using Duncan's multiple range test (DMRT).

## Results

### Isolation, identification and characterization of the PGPR isolates

The PGPR obtained from rhizosphere of coconut from Kidu located in Karnataka, India was designated as KiSII and that of Ratnagiri, Maharashtra, India as RNF 267. Both of them were identified as gram negative motile rods producing smooth and shiny colonies on KBA. Additionally, KiSII showed pink colored pigment on KBA. The biochemical characterization revealed these isolates belonged to *Enterobacteriaceae* family and based on further tests, the isolates KiSII and RNF 267 were identified as *Serratia marcescens* and *Enterobacter cloacae*, respectively (Table 1). Biolog provided the identification of KiSII as *Serratia marcescens* ss *marcescens* with similarity index 0.617 and distance 5.087 (99.3 % probability), validating the conventional identification. Biolog assay of RNF 267 gave similarity index (0.046) lower than the acceptable value (0.5), though it showed maximum similarity with *Enterobacter cloacae* in the database. The molecular analysis based on 16S rDNA gene homology identified the PGPR KiSII as *Serratia marcescens* and RNF 267 as *Enterobacter* sp. with 99 and 97 % similarity with the respective strains in NCBI GenBank database. The nucleotide sequences were deposited in NCBI GenBank database under accession numbers JX498910 (*S. marcescens*) and JX498911 (*Enterobacter* sp.).

The PGPR isolate *Serratia marcescens* KiSII was found to be growing at a temperature range of 4–40 °C and showed resistance to 6 % NaCl concentration and it could grow in the liquid medium having pH range of 4.2–9.0. In contrast to this, *Enterobacter* sp. RNF 267 could grow in temperature range of 15–40 °C and pH range of 4.2–9.0, and could tolerate up to 8 % NaCl concentration.

**Table 1** Morphological, biochemical and physiological characters of bacterial isolates KiSII and RNF 267

| Characteristic (s) | KiSII | RNF 267 |
|---|---|---|
| Gram reaction | − | − |
| Cellular Morphology | Short rod | Short rod |
| Motility | + | + |
| Oxidase reaction | − | − |
| VP reaction | + | + |
| Arginine dihydrolase | − | + |
| Gelatin hydrolysis | + | − |
| Nitrate reduction | + | + |
| Indole production | − | − |
| D-glucose (acid/gas) | +/no gas | +/Gas |
| Sucrose (acid/gas) | +/no gas | +/Gas |
| Sorbitol (acid/gas) | +/no gas | +/Gas |
| Inositol (acid) | + | − |
| Mannitol (acid) | + | + |
| Trehalose (acid) | + | + |
| Arabinose (acid) | − | + |
| Xylose (acid) | − | + |
| Glycerol (acid) | + | + |
| Pigment (red) | + | − |
| Tentatively identified as | *S. marcescens* | *E. cloacae* |

### Assays for plant growth promotion traits

The PGPR isolate *S. marcescens* KiSII possessed all the plant growth promotion traits tested, whereas *Enterobacter* sp. RNF 267 was negative for some traits (Table 2). Chitinase activity was detected only in *S. marcescens* KiSII and it showed 36 units of enzyme activity in liquid culture. A maximum of 216 µg/ml of phosphorus was released by *S. marcescens* KiSII in 72 h of incubation, whereas *Enterobacter* sp. RNF 267 recorded the peak soluble phosphorus content (217 µg/ml) at 48 h. Quantitative determination of $\beta$-1,3-glucanase activity revealed that *S. marcescens* KiSII and *Enterobacter* sp. RNF 267 produced 14.75 and 20.33 units respectively.

### Green house assay

The results of green house assay revealed that *S. marcescens* KiSII and *Enterobacter* sp. RNF 267 significantly increased the plant growth parameters of paddy seedlings and cowpea seedlings (Tables 3, 4). Dry matter increased up to 60 and 100 % in paddy seedlings and 104 and 48 % in cowpea seedlings inoculated with *S. marcescens* KiSII

Table 2 Plant growth promotion traits possessed by bacteria KiSII and RNF 267

| Sl. No | Plant growth promotion traits | KiSII | RNF 267 |
|---|---|---|---|
| 1 | IAA production | 2.88 µg/ml | 2.40 µg/ml |
| 2 | Phosphate solubilization | 216 µg/ml[a] | 217 µg/ml[b] |
| 3 | Growth on N-free medium | + | − |
| 4 | ACC-deaminase production | + | + |
| 5 | Chitinase activity* | 36 U | − |
| 6 | $\beta$-1,3 glucanase activity** | 15 U | 20 U |
| 7 | Siderophore production | + | − |
| 8 | Antibiotic production | + | − |
| 9 | Ammonia production | + | + |

*One unit (U) of chitinase activity was defined as the amount required releasing µg of N-acetyl glucosamine from chitin per hour per mg protein

**One unit (U) of glucanase activity was defined as the amount required releasing µg of glucose from laminarin per minute per mg protein

[a] Maximum P-solubilization at 72 h

[b] Maximum P-solubilization at 48 h

and RNF 267, respectively. There was no influence on the total seedling length and fresh weight of paddy seedlings inoculated with *S. marcescens* KiSII. On the other hand, the paddy seedlings inoculated with *Enterobacter* sp. RNF 267 showed an increase in all the parameters like seedling length (13 %), fresh weight (51 %) and dry weight (100 %) over uninoculated control. The growth parameters of cowpea seedlings were highly influenced by bacterization with *S. marcescens* KiSII and *Enterobacter* sp. RNF 267, and were statistically significant at $P = 0.05$.

## Poly bag assay on coconut seedlings

Bacterial inoculation of coconut seedlings resulted in significant increase in leaf length, shoot length, root length and seedling length (Fig. 1). Maximum seedling length was induced by *S. marcescens* KiSII (174.0 cm). It was significantly different from the untreated control at $P = 0.05$. And highest leaf length was also recorded in coconut seedlings treated with *S. marcescens* KiSII (109.78 cm). Fresh weight of shoot and root did not differ significantly in all treatments (Fig. 2). However it was relatively superior in inoculated treatments. Seedling dry weight was significantly higher in *Enterobacter* sp. RNF 267 (164.10 g) followed by *S. marcescens* KiSII (163.58 g) and untreated control (137.47 g). Seedling girth was statistically higher in *Enterobacter* sp. RNF267 (14.81 cm) (Table 5). The seedlings inoculated with *Enterobacter* sp. RNF 267 produced maximum leaves (7.20) followed by *S. marcescens* KiSII (7.19). Also, there was an increase in the uptake of the three major nutrients noticed in the bacterized seedlings (Table 6). The coconut seedlings inoculated with PGPR *S. marcescens* KiSII recorded highest N (2.25 g/plant) and P uptake (103.3 mg/plant) and the values were statistically ($P = 0.1$) on par with *Enterobacter* sp. RNF267. Whereas the highest K uptake (1.96 g/plant) was recorded in seedlings inoculated with *Enterobacter* sp. RNF267 which was significantly superior over other treatments.

Table 3 Effect of bacterium KiSII on growth of test plants in green house conditions

| Growth parameter(s) | Paddy | | Cowpea | |
|---|---|---|---|---|
| | Control | Inoculated | Control | Inoculated |
| Root length (cm) | 13.98 (±1.2) | 14.83 (±1.9) | 19.64 (±0.4) | 21.17 (±0.4)* |
| Shoot length (cm) | 29.73 (±1.1) | 28.86 (±1.2) | 44.93 (±1.5) | 52.51 (±1.2)* |
| Total seedling length (cm) | 43.70 (±1.4) | 43.70 (±1.6) | 64.57 (±1.6) | 73.68 (±1.4)* |

Values are an average of ten replications ± standard error

* Significant at $P = 0.05$

Table 4 Effect of bacterium RNF 267 on growth of test plants in green house conditions

| Growth parameter | Paddy | | Cowpea | |
|---|---|---|---|---|
| | Control | Inoculated | Control | Inoculated |
| Root length (cm) | 6.38 (±0.5) | 7.07 (±0.6) | 19.28 (±0.9) | 21.82 (±0.7)* |
| Shoot length (cm) | 15.78 (±0.9) | 18.03 (±1.1) | 37.06 (±1.7) | 42.85 (±1.3)* |
| Total seedling length (cm) | 22.15 (±1.1) | 25.10 (±1.3) | 56.35 (±2.0) | 64.67 (±1.5)* |

Values are an average of ten replications ± standard error

* Significant at $P = 0.05$

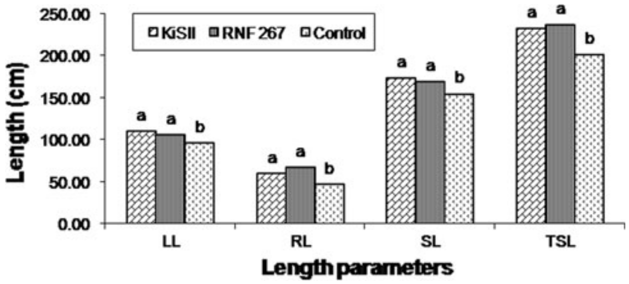

**Fig. 1** Effect of bacterial inoculations on length parameters of coconut seedlings. *LL* leaf length, *RL* root length, *SL* shoot length, *TSL* total seedling length. Lengths (cm) are mean of twenty replications per treatment. *All bars* having the *same letters* are not different ($P = 0.05$) from each other by Duncan's multiple range test

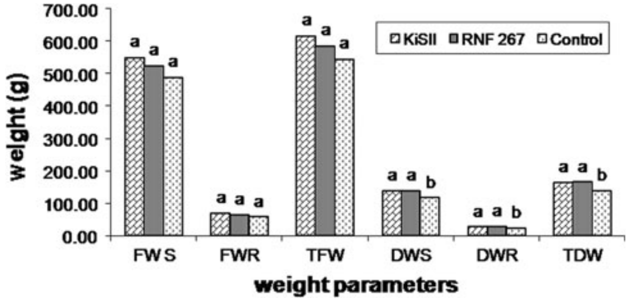

**Fig. 2** Effect of bacterial inoculations on weight parameters of coconut seedlings. *FWS* fresh weight of shoot, *FWR* fresh weight of root, *TFW* total fresh weight of root and shoot, *DWS* dry weight of shoot, *DWR* dry weight of root, *TDW* total dry weight of root and shoot. *All bars* having the *same letters* are not different ($P = 0.05$) from each other by Duncan's multiple range test. Values are mean of 20 replications

**Table 5** Effect of bacterial inoculations on leaf number (LN), leaf width (LW), seedling girth (GS), root number (RN) of coconut seedlings

| Treatment | LN | LW (cm) | GS (cm) | RN |
|---|---|---|---|---|
| KiSII | $7.19^{a}$ | $22.44^{a}$ | $14.79^{a}$ | $21.44^{a}$ |
| RNF 267 | $7.20^{a}$ | $19.90^{b}$ | $14.81^{a}$ | $17.23^{a}$ |
| Control | $6.60^{b}$ | $20.65^{b}$ | $13.93^{b}$ | $17.20^{a}$ |

Means followed by the same letter in a column do not different significantly according to Duncan's multiple range test at $P = 0.05$

**Table 6** Effect of bacterial inoculations on nutrient uptake of coconut seedlings

| Treatment | N uptake (g/plant*) | P uptake (mg/plant*) | K uptake (g/plant*) |
|---|---|---|---|
| KiSII | $2.25^{a}$ | $103.3^{a}$ | $1.74^{b}$ |
| RNF 267 | $2.13^{a}$ | $102.0^{a}$ | $1.96^{a}$ |
| Control | $1.46^{b}$ | $59.3^{b}$ | $1.02^{c}$ |

All columns having the same letters are not different from each other by Duncan's multiple range test ($P = 0.01$)

* Above ground level

Besides this, there was a significant increase in population of beneficial microbial flora such as *Bacillus* spp., fluorescent pseudomonads, phosphate solubilizers and nitrogen fixers in the rhizosphere of coconut seedlings (Fig. 3). Fluorescent pseudomonads were not detected in uninoculated control.

## Discussion

The PGPR KiSII, isolated from the rhizosphere of coconut growing in Kidu, Karnataka, India was identified as *S. marcescens* by conventional biochemical tests, Biolog tests and 16S rDNA sequence analysis. Conventional tests suggested RNF 267 to be *E. cloacae* while Biolog provided no ID. However, 16S rDNA sequence analysis proved it as *Enterobacter* sp. Biolog systems software is used to identify the bacterium from its phenotypic pattern in the Gen III Microplate. *Enterobacter* sp. RNF 267 showed the maximum similarity index with *Enterobacter cloacae* in the Biolog database and the isolate differed from *E. cloacae* in non-utilization of D-sorbitol as carbon source. Biolog is a relatively simple and widely used method to assess the functional diversity of microorganisms. It analyses the ability of the cell to metabolize all major classes of biochemicals, in addition to determining other physiological properties such as pH, salt, reducing power and chemical sensitivity (http://www.biolog.com). Though *Serratia* and *Enterobacter* are in the same family, there were large differences in the carbon utilization profile. Among the 71 carbon sources, 63 were utilized by *S. marscens* KiSII. It showed a high oxidation capacity for L-histidine ($OD_{590}$ 1.647) followed by N-acetyl-D-glucosamine ($OD_{590}$ 1.374). *Enterobacter* sp. RNF 267 used 51 of the 71 carbon sources with maximum colour development in the well containing

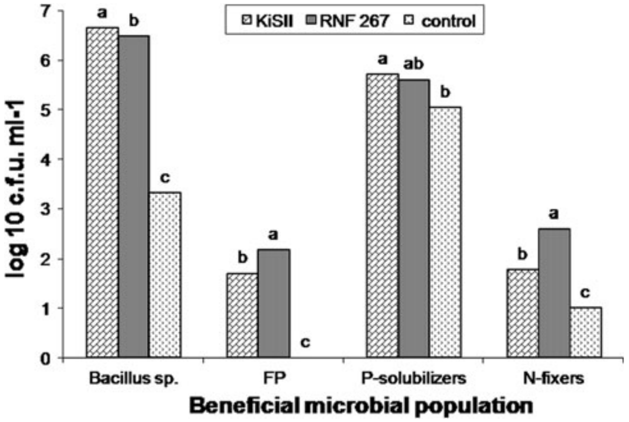

**Fig. 3** Effect of bacterial inoculations on distribution of plant beneficial microbes in rhizosphere of coconut seedlings. *FP* fluorescent *Pseudomonas* spp. *All bars* having the *same letters* are not different ($P = 0.05$) from each other by Duncan's multiple range test

N-acetyl-D-glucosamine ($OD_{590}$ 1.592). It is possible to give introduced bacterial strains a competitive advantage by providing them with a substrate that they can readily utilize as a carbon source via a seed inoculation formulation (Cakmakci et al. 2010). The high utilization rates of raffinose, lactose, glucose, fructose, serine, glutamine and alanine, which are commonly present in coconut root exudates (Bopaiah et al. 1987), by these isolates, relate to the colonization of roots by bacteria (Alisi et al. 2005). Abiotic stress tolerance studies revealed that both the strains could tolerate wide range of temperature (4/15–40 °C), salinity (6–8 %) and pH (4–9). It is reported that the ability to withstand the adverse environmental conditions is significant not only for rhizobacterial survival in tropical agricultural soils but also in their utility as biofertilizer (Banarjee et al. 2010).

Three approaches were employed to determine the plant growth promoting potential of *S. marcescens* KiSII and *Enterobacter* sp. RNF 267. This included in vitro testing of plant growth promotion attributes, green house assay and polybag assay. Both the bacteria exhibited phosphate solubilization, ammonification, production of indole acetic acid (IAA), $\beta$-1,3 glucanase activity and ACC-deaminase activities in the in vitro assays. In addition, the strain *S. marcescens* KiSII exhibited N- fixation potential, production of siderophore, antibiotics and chitinase activities. Indian soils are normally deficient in available phosphorus even though the bound component may be sufficiently abundant (Johri et al. 2003); therefore, the use of phosphate solubilizing bacteria is very common. It was reported that the application of phosphate solubilizing bacterium *Bacillus* M-13 could mobilize phosphorus efficiently in sunflower and improved seed quality and oil yield (Ekin 2010). Rhizosphere bacteria are known to enhance root growth and development of plants through the synthesis of the plant auxin IAA (Taghavi et al. 2010) and ACC deaminase (Ghosh et al. 2003). The role of lytic enzymes appears to be significant in biological control because chitin and laminarin are present in the cell wall of many fungal pathogens. The lytic enzymes $\beta$-1,3-glucanases and chitinases produced by *Trichoderma longibrachiatum* responsible for the degradation of *Thielaviopsis* hyphae had been reported (Sanchez et al. 2007). The capability of the rhizobacteria to fix atmospheric nitrogen was detected in PGPR isolate *S.marcescens* KiSII as evidenced by the thick growth on Jensen's agar. Isolation and identification of nitrogen fixers have been reported from the rhizosphere of wild raspberries which were able to grow in N-free medium and showed nitrogenase activity (Cakmakci et al. 2008). Siderophore production is another important trait of the PGPRs that influences plant growth through the suppression of fungal pathogens by rendering iron unavailable by binding in the rhizosphere (Rahi et al. 2009). In our study, presence of siderophore was detected in the PGPR isolate *S. marcescens* KiSII. These are some of the direct and indirect mechanisms by which rhizobacteria are thought to enhance the plant growth (Saharan and Nehra 2011) and the results suggested that bacteria possessing these traits might increase the plant growth. Hence, these isolates were subjected to plant tests in short duration test crops (paddy and cowpea) as well as coconut seedlings.

In our study, both the PGPRs increased the root length, shoot length, and total dry matter of all the test plants over the un-inoculated control. Increase in dry biomass of paddy seedlings at 21 days after sowing was 60 % and 100 % with *S. marcescens* KiSII and *Enterobacter* sp. RNF 267, respectively, under green house conditions. Dry weight of cowpea seedlings increased by 104 and 48 %, respectively, for *S. marcescens* KiSII and *Enterobacter* sp. RNF 267, over control. The inoculation in coconut seedlings also significantly enhanced growth and nutrient uptake under the field conditions. Increase in coconut seedling biomass (total dry weight) was 19 % with *S. marcescens* KiSII and *Enterobacter* sp. RNF 267 when compared to uninoculated control. The PGPRs showed significant increase in the collar girth of the seedlings, and according to Ramadasan et al. (1985) girth at collar region of coconut seedling is an important character which determines the seedling vigour. More important in seedling development and outplanting success is root health and root architecture, which have been shown to greatly influence seedling survival after outplanting (Wisniewski et al. 1991). In this study, the root biomass of coconut seedling increased with PGPR inoculation accompanied with significant increase in the length. An incremental effect on growth observed in paddy, cow pea and coconut seedlings indicated plant growth promoting activity by these isolates in a broad host range. Recently, *Serratia marcescens* (TRS-1) was reported to promote growth in tea seedlings as evidenced by increase in height, emergence of new leaves and branches, as well as increase in leaf biomass (Chakraborty et al. 2010). In an earlier report, seed bacterization with *Serratia marcescens* strain SRM (MTCC 8708) isolated from flowers of summer squash, significantly enhanced plant biomass of wheat seedlings grown in cold temperatures (Selvakumar et al. 2007). Species of *Enterobacter* are also reported to enhance plant growth in *Brassica oleracea* (Zakria et al. 2008). *Enterobacter* sp. 638 was able to increase the growth of several species of poplar by up to 40 % (Taghavi et al. 2010). Plant growth and development by this isolate seemed to rely on the production of indole acetic acid (IAA), solubilization of phosphates, ACC deaminase production. This study corroborate our results as our isolates possessed important plant growth promotion traits and enhanced growth of short duration test crops and coconut seedlings as well. Significant increase in root length of the test plants might have been due to the production of IAA and ACC deaminase activity by the isolates. The ability of the isolates to grow in nitrogen free conditions, to solubilize insoluble minerals and to increase root length could have resulted in increased uptake of major nutrients by the seedlings. Inoculation of PGPR isolates *S. marcescens* KiSII and *Enterobacter* sp. RNF 267 could promote plant-beneficial bacteria in the rhizosphere region of coconut seedlings, which in turn could have aided in enhanced growth of the coconut seedlings. In inorganic farming a modification of natural balance can drastically alter the microbial community leading to loss of beneficial microbes and an ingress of plant pathogens which may have a devastating effect on plant productivity (Avis et al. 2008). Such detrimental soil microbial changes can be prevented by use of these types of bio-inoculants. All these activities by the PGPR isolates go a long way in better nutrient mobilization, availability, and thus enhanced growth and development of the coconut seedlings. In conclusion, the bacteria *S. marcescens* KiSII and *Enterobacter* sp. RNF 267 isolated from coconut rhizosphere were observed to possess multifarious plant growth promotion traits. Upon their inoculation, the coconut seedlings grew more vigorously accompanied by better nutrient uptake and increased plant-beneficial microbial community population in their rhizosphere. Seedlings biporimed with PGPRs are likely to grow better than the uninoculated ones when they are transplanted in the field. Utilization of these microorganisms would enable reduced use of pesticides and fertilizers that are potential pollutants of the environment and thus to achieve sustainable organic farming of coconut.

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