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Isolation, characterization, and evaluation of multi-trait plant growth promoting rhizobacteria for their growth promoting and disease suppressing effects on ginger

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ABSTRACT

In this study, 100 PGPR strains isolated from different varieties of ginger (*Zingiber officinale* Rosc.) were first characterized for their morphological, biochemical, and nutrient mobilization traits *in vitro*. The PGPR were also screened *in vitro* for inhibition of *Pythium myriotylum* causing soft rot in ginger. Results revealed that only five PGPR showed >70% suppression of *P. myriotylum*. These 5 PGPR *viz*., GRB (Ginger rhizobacteria) 25 – *Burkholderia cepacia*, GRB35 – *Bacillus amyloliquefaciens*; GRB58 – *Serratia marcescens*; GRB68 – *S. marcescens*; GRB91 – *Pseudomonas aeruginosa* were used for further growth promotion and biocontrol studies in the green house and field. The green house study revealed that GRB35 (*B. amyloliquefaciens*) and GRB68 (*S. marcescens)* registered markedly higher sprouting (96.3%) and lower disease incidence (48.1%) and greater rhizome yield (365.6 g pot^{-1} and 384.4 g pot^{-1}, respectively), while control registered the lowest sprouting (66%), maximum soft rot incidence (100%) and lowest rhizome yield (134.4 g pot^{-1}). In the field experiments also, GRB68 (*S. marcescens*) and GRB35 (*B. amyloliquefaciens*) registered the greatest sprouting (80% each), markedly lower soft rot incidence (5.2% and 7.3%, respectively) and higher yield (5.0 and 4.3 kg^3 m^{-2}, respectively) compared to chemicals like Streptomycin sulphate (73.0%, 18.5% and 2.3 kg^3 m^{-2}, respectively), Metalaxyl–Mancozeb (73.0%, 14.0% and 3.8 kg^3 m^{-2}, respectively) and control (73.0%, 25.1% and 2.2 kg 3 m^{-2}, respectively). Overall, the results suggested that for growth promotion and management of soft rot disease in ginger, GRB35 *B. amyloliquefaciens* and GRB68 *S. marcescens* could be good alternatives to chemical measures. Since, the latter has been reported to be an opportunistic human pathogen, we recommend the use of *B. amyloliquefaciens* for integration into nutrient and disease management schedules for ginger cultivation.

1. Introduction

Plant growth promoting rhizobacteria (PGPR) represent a wide variety of rhizosphere-inhabiting bacteria which colonize the root systems of plants and can stimulate plant growth by direct or indirect mechanisms. Direct mechanisms of plant growth promotion include biofertilization, stimulation of root growth, rhizoremediation, and plant stress control, while mechanisms of biological control include reducing the level of disease, antibiosis, induction of systemic resistance, and competition for nutrients and niches (Lugtenberg and Kamilova 2009). In other words, the PGPR can stimulate plant growth, increase yield, reduce pathogen infection, as well as reduce biotic or abiotic plant stress, without conferring pathogenicity (Adesemoye and Kloepper 2009; van Loon and Bakker 2005). Common PGPR include the strains in the genera *Acinetobacter*, *Alcaligenes*, *Arthrobacter*, *Azospirillum*, *Azotobacter*, *Bacillus*, *Beijerinckia*, *Burkholderia*, *Enterobacter*, *Erwinia*, *Flavobacterium*, *Rhizobium* and *Serratia* (Anandaraj and Dinesh 2008). At present, PGPR are being increasingly used in combination with manures and fertilizers for improving crop yields and have contributed to the development of sustainable agricultural systems.

Studies have shown that PGPR had positive effects on cereals (Shaharoona et al. 2006), fruits (Kavino et al. 2010), vegetables (Kurabachew and Wydra 2013), flowers (An et al. 2010) and spices like black pepper (Diby and Sarma 2006). PGPR have also been found to improve the use efficiency of applied fertilizers and

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manures thus allowing reduced application rates (Adesemoye et al. 2009). PGPR have also been found to enhance the biological quality of soils through enhanced microbial and enzyme activities (Dinesh et al. 2013).

Besides, in addition to growth promotion, there are numerous studies that have reported the biocontrol potential of PGPR in agriculture. Recently, Son et al. (2014) found that among selected PGPR isolates, four significantly decreased gray leaf spot disease severity with PGPR *Brevibacterium iodinum* KUDC1716 providing the highest disease suppression in pepper (*Capsicum annuum*). It was also found that *P. polymyxa* increased plant growth of pepper (*C. annuum*) by decreasing the severity of *Xanthomonas axonopodis* pv. *Vesicatoria* (Quyet-Tien et al. 2010). In another green house study, Dinesh et al. (2014) found lowest root rot and taller plants by the application of *Burkholderia cepacia* BRB 21 in black pepper. Out of the 47 PGPR isolated from several monocotyledons, several of them were found to have highly inhibitory effects against *F. oxysporum radicis-lycopersici*, *Sclerotium bataticola*, *Pythium ultimum* and *F. graminearum* (Laslo et al. 2012). Thus, PGPR are being widely employed to attenuate the ill effects of inappropriate agricultural intensification and reckless use of chemicals. In fact, the use of PGPR is considered a benign environment friendly practice that lays emphasis on restoration and maintenance of environment both in the short- and long-term.

It is, therefore, apparent that numerous reports are available on isolation of PGPR and their effects on growth and yield of an array of crops. However, very little information exists on the effects of native multitrait PGPR on growth promotion of ginger cultivated as a rainfed crop in high rainfall zones of the humid tropics. Ginger (*Zingiber officinale* Rosc. Family: *Zingiberaceae*) is an herbaceous perennial, the rhizomes of which are used as a spice. India is a leading producer of ginger in the world and during 2012–13 the production is estimated to be 0.754 mt from an area of 0.158 mha. Ginger is cultivated in most of the states in India. However, states namely Kerala, Meghalaya, Arunachal Pradesh, Mizoram, Sikkim, Nagaland and Orissa together contribute 70% to the country's total production. Ginger thrives best in well drained soils like sandy loam, clay loam, red loam or lateritic loam. A friable loam rich in humus is ideal. Our objectives in this study conducted from 2006 to 2014 were to isolate PGPR from ginger, characterize them and shortlist those with maximum plant growth promotion (PGP) traits and study their effects on ginger rhizome sprouting and yield through green house and field experiments. Another objective was to determine the effects of the promising PGPR on inhibition of the soft rot causing pathogen, *Pythium myriotylum*.

2. Materials and methods

2.1. Soil sampling

For isolation of PGPR, soils were collected from the rhizosphere of 12 varieties of ginger *viz*., PGS, E2V52, *Varada*, *Mahima*, *Himachal*, *Suprabha*, *Maran*, *Rejatha*, *Dhanja*, *Mizoram*, RARS-1 and *Rio-de Janeiro* grown in Calicut district of Kerala state (India) and Kodagu District of Karnataka state (India). The soils strongly adhering to the roots and within the space explored by the roots were considered as the rhizosphere soil (Garcia et al. 2005). Soil samples were collected from randomly selected ginger crop under each cultivar. The soils were immediately transferred to ice box for transport. In the laboratory, the living plant material and coarse roots were removed prior to estimation of moisture content in the samples. A portion of each sample required for estimating the biochemical/microbial parameters was stored at 4 °C for not more than one week before analyses.

2.2. Isolation of PGPR

Exactly 10.0 g moist soil was placed in 95 mL dilution blank, sterile water (10^{-1}), shaken for 10 min. Then, 1.0 mL of this suspension was transferred into a 9 mL blank (10^{-2}). This serial dilution was continued up to 10^{-10}, followed by pour plating on different types of medium *viz*., Tryptic Soy Agar (TSA), Nutrient Agar (NA), basal medium amended with glucose, mannitol, sorbitol, inositol and sucrose. The plates were incubated at 28 °C for 2–3 days. Maximum diversity and abundance of rhizobacteria were present in Tryptic Soy Agar (TSA) followed by Nutrient Agar (NA). The most suitable dilution was selected for estimating the population of rhizobacteria, and expressed as number of CFU (Colony Forming Units) g^{-1} soil. The individual bacterial colonies were then selected and subcultured on nutrient agar. A total of 100 isolates thus obtained were cryopreserved at −80 °C in 40% glycerol for further studies.

All the isolates were studied for their morphological characters *viz*., cell form and size, Gram staining, spore formation, motility, colony pigmentation and production of UV-fluorescent pigments. The biochemical traits studied were indole test, methyl red test, Voges-Proskauer (VP) test, citrate test, presence of oxidase and catalase, succinic acid, carbohydrate utilization pattern, hydrolysis of casein, starch, pectin production of UV-fluorescent pigments, citrate, presence of oxidase and catalase, succinic acid, casein hydrolysis, starch hydrolysis and ammonia production. The growth at varying temperatures (28 °C, 37 °C, 41 °C, 50 °C and 60 °C) and salt concentrations (1%, 2%, 5%, 7% and 10% NaCl) were also studied.

2.3. Phenotypic identification of PGPR isolates

All the isolates were tentatively grouped based on phenotypic identification such as cell form and size; Gram staining; spore formation; motility; colony pigmentation; production of UV-fluorescent pigments; indole; methyl red; VP; citrate; the presence of oxidase and catalase, succinic acid, casein hydrolysis, starch hydrolysis and ammonia production, growth at 28 °C, 37 °C, 41 °C, 50 °C and 60 °C and growth at 1%, 2%, 5%, 7% and 10% salt concentration were assessed for each rhizobacteria as described by Holt et al. (1994) and Tindal et al. (2007).

2.4. Screening for growth promoting traits and hydrolytic enzymes

2.4.1. Production of indole acetic acid (IAA), ammonia (NH_3) and hydrogen cyanide (HCN)

The isolates were tested for their ability to produce IAA by the method of Sawar and Kremer (1995). The test organism was inoculated by adding 50 μL of cell suspension in 5 mL of the sterile tryptone/peptone yeast extract broth (peptone/tryptone – 10 g, beef extract – 3 g, NaCl – 5 g, L-tryptophan – 0.204 g, distilled water – 1 L; pH – 7) into 15 mL culture tubes and incubated for 72 h at 28 °C in the dark. Subsequently, 1.5 mL of this broth was centrifuged at 12,850 × *g* for 10 min, followed by addition of 1 mL of Salkawaski reagent (50 mL, 35% of perchloric acid, 1 mL 0.5 M $FeCl_3$ solution) to the 1 mL of the supernatant in 2 mL eppendorf tube. The culture tubes were then incubated at 37 °C in the dark for 1 h. Formation of red color in the medium indicated IAA production by the organism.

NH_3 production was determined by the method described by Cappuccino and Sherman (1992). Briefly, 50 μL of bacterial cell suspension was inoculated in 30 mL of peptone broth (4%) and incubated at 25 °C for 72 h. After the incubation, 1 mL Nessler's reagent (potassium iodide – 50 g, saturated mercuric chloride – 35 mL, Distilled water – 25 mL, potassium hydroxide (40%) – 400 mL) was added. The formation of yellow to brown precipitate showed the presence of NH_3.

Production of HCN was measured by the qualitative method of Kloepper et al. (1991) with few modifications. Log phase cultures (25 μL) of the bacteria were inoculated into 5 mL of King's B broth supplemented with 4.4 g L^{-1} of glycine taken in 30 mL sterile glass vials. Filter paper strips soaked in picric acid solution (2.5 g picric acid + 12.5 g Na_2CO_3 in 1 L distilled H_2O) were inserted in half of the vials and tightened with screw cap. Vials were sealed with parafilm and incubated for 72 h in a mechanical shaker. Production of HCN was indicated by the change in color of the filter paper strips from yellow to brown to red. The intensity of the color was recorded visually.

2.4.2. Solubilization of P, K, Zn and Si

Solubilization of P was tested using Pikovskaya's agar medium (glucose – 10 g, calcium phosphate – 5 g, ammonium sulphate – 0.5 g, potassium chloride – 0.2 g, magnesium sulphate – 0.1 g, yeast extract – 0.5 g, agar – 15 g, distilled water – 1000 mL). One loop full of the 24 h broth culture was spot inoculated on the Pikovsakya culture plate. The plates were incubated at 28 °C for 96 h. The plates were observed for the zone of clearance around the bacterial colony, which indicated solubilization of P. The solubilization zone was determined by subtracting the diameter of bacterial colony from the diameter of total zone (Gaur 1990).

For determining K solubilization, the cultures were inoculated onto Aleksandrov medium containing mica (Hu et al. 2006). The plates were incubated at room temperature (30 ± 1 °C) for 3 days and the colonies exhibiting clear zones were selected and diameter of solubilization zone was measured. Zn solubilization was determined in mineral salts agar medium (Glucose – 10 g, $(NH_4)_2SO_4$ – 1.0 g, KCl – 0.2 g, K_2HPO_4 – 0.1 g, $MgSO_4$ – 0.2 g and H_2O – 1000 mL with pH 7.0) amended with 0.1% of insoluble zinc oxide (ZnO) or zinc carbonate ($ZnCO_3$). The actively growing cultures (5 μL) were spot inoculated onto the medium, incubated at 28 °C and solubilization zone was measured 15 days after inoculation and clearing zone was expressed as area in cm^2 (Venkatakrishnan et al. 2003).

For measuring Si solubilization, the test media was prepared by adding 5 g potassium alumina silicate ($KAlSi_3O_8$) in the basal media after sterilization. Then the media was poured in to petri plates and 0.5 cm diameter well was made after solidification, following which 0.1 mL of the bacterial suspension was added to each well and incubated at 28 °C for 4–5 days. The plates were observed for zone of clearance around the wells (Barker et al. 1998).

2.4.3. Hydrolytic enzymes production

Production of cell-wall-degrading enzymes (α-amylase, cellulase, pectinase and protease) is a common mechanism used by bacteria to inhibit growth of the other microorganisms. This was measured by the methods described by Cappuccino and Sherman (1992). For determining the production of α-amylase and protease enzymes, one loopful of the bacterial cell suspension was streaked on starch agar plate (peptone – 5 g, beef extract – 3 g, soluble starch – 10 g, agar – 15 g, distilled water – 1000 mL) and skim milk agar plate (skim milk – 100 g, peptone – 5 g, agar – 15 g, water – 1000 mL), respectively. After 48 h of incubation at 28 °C, the plates were observed for clear zone around the streak.

For determining pectinase and cellulase production, the media was prepared by adding 1% pectin and cellulose in basal medium ($NaNO_3$ – 1 g, K_2HPO_4 – 1 g, KCl – 1 g, $MgSO_4$ – 0.5 g, yeast extract – 0.5 g, glucose – 1 g, distilled water – 1000 mL, Agar – 15 g). One loopful of the bacterial cell suspension was streaked on the medium and incubated for 5 days. Gram's iodine solution was poured in the pectin agar and zone of clearance was observed against the dark blue back ground. The cellulose medium was flooded with 0.01% congo red solution for 15 min and the plates were destained using 1% NaCl solution for 5 min. A clear zone against the red back ground indicated that the rhizobacteria was positive for pectinase and cellulase production.

2.5. In vitro screening of rhizobacteria for antagonism against P. myriotylum

All the 100 PGPR were screened using dual plate culture technique (Berg et al. 2002) against pathogenic fungi *P. myriotylum* Drechs., causing soft rot in ginger. The test was done in three independent replicates with *P. myriotylum* (isolated from ginger) on Potato Dextrose Agar (PDA). Briefly, a mycelial plug of actively growing pathogen *was* placed on to the center of the respective agar medium and the PGPR strain was streaked 2 cm away on either side of mycelial plug. Plates were then incubated at 28 °C for about 5 days or until the leading edge of fungus in the control plate reached the edge of the plate. The radial growth of fungal mycelium was measured and percent inhibition of growth over untreated control was estimated and the percentage inhibition was compared with control using the formula,

$$I = [C - T/C] \times 100 \quad (1)$$

where I is the per cent inhibition and C and T are the radial growth of the pathogen in control and treatment, respectively. Rhizobacterial isolates that showed more than 50% inhibition against any of the three pathogens were short-listed.

2.6. Identification of bacterial isolates

The preliminary identification of the isolates was done using the Bergey's Manual of Determinative Bacteriology and the identity of the 8 promising PGPR (Table 1) with multiple PGP traits (GRB25 – *B. cepacia*, GRB35 – *Bacillus amyloliquefaciens*; GRB38 – *Serratia marcescens*; GRB58 – *S. marcescens*; GRB68 – *S. marcescens*; GRB70 – *Enterobacter aerogenes*; GRB71 – *Enterobacter aerogenes* and GRB91 – *Pseudomonas aeruginosa*) was confirmed using Biolog Microstation System (RDG Laboratories, Hayward, California, USA), wherein the single colony of test organism was inoculated into IFA – GEN III inoculation fluid. After confirming the uniform distribution of bacterial cells (turbidity 1 in Biolog turbidometer), 500 μL of this inoculation fluid was loaded in GEN III plates. After 24 h of incubation, the plate was read in Biolog Microstation System (Version 5.1.1) using GEN III software.

The identity of the two most promising PGPR (GRB35 – *B. amyloliquefaciens* and GRB68 – *S. marcescens*) was reconfirmed using 16S rDNA sequence analysis. Briefly, the genomic DNA from the short listed PGPR using the standard protocol (Sambrook and David 2000). The 16S rDNA gene was amplified using universal primer set (Woese 1987; Stackebrandt and Goebel 1994), pA (5′-AGAGTTTGATCCTGGCTCAG-3′) and pH (5′-AAGGAGGTGATCCAGCCGCA-3′) in 25 μL of reaction mixture containing 1× buffer (10 mM Tris pH 9, 50 mM KCl, 0.01% gelatin), 100 μm dNDP's mix, 3 mM $MgCl_2$, 10 μg BSA, 5pM each primer, 0.5 units of Taq DNA polymerase and 100 ng template DNA. The thermo cycling condition consisted of an initial denaturation at 94 °C for 1 min 10 s, 48 °C for 30 s, 72 °C for 2 min 10 s and a final polymerization step of 72 °C for 6 min with Eppendorf master thermal cycler. The final PCR product was resolved in 0.8% agarose gel in Tris acetate EDTA buffer at 4 V cm^{-1}. The PCR product was excised and purified with elution kit (Sigma, India). The DNA sequencing was performed with Chromos Biotech, Bengaluru (India). Sequence was subjected to BLAST analysis and compared with registered sequences in the GenBank database using NCBI Blast server (http://www.ncbi.nlm.nih.gov). BLAST analysis showed 99–100% similarity with 16S rRNA gene sequences of *B. amyloliquefaciens* already available in GenBank. Same was the case with *S. marcescens*. The 16S rDNA sequences of these two PGPR were deposited in the

Table 1
Shortlisted rhizobacteria from ginger with multiple plant growth promotion traits *in vitro*.

Isolate[a]	Hydrolytic enzyme production				Growth promoting traits		Nutrient solubilization traits				HCN production
	Protease	Cellulase	Pectinase	α-amylase	Indole acetic acid (IAA)	NH_3 production	P	Zn	K	Si	
GRB25	–	–	–	–	+	+	–	+	+	++	–
GRB35	+	+	+	+	++	+	+	+	+	+	+
GRB38	–	–	–	–	+++	+	+	++	–	+	–
GRB58	+	–	–	–	+	+	–	–	–	–	–
GRB68	+	–	–	–	+	+	+	+	–	–	+
GRB70	++	–	–	–	++	+	++	–	+	–	–
GRB71	+++	–	–	–	+	+	–	+	+	+	–
GRB91	++	+	–	+	–	–	+	+	–	–	–

[a] GRB25 – *Burkholderia cepacia*; GRB35-Bacillus amyloliquefaciens; GRB38 – Serratia marcescens; GRB58 – Serratia marcescens; GRB68 – Serratia marcescens; GRB70 – Enterobacter aerogenes; GRB71 – Enterobacter aerogenes; GRB91 – *Pseudomonas aeruginosa*.

GenBank database of NCBI. The GenBank accession numbers are FJ493538 for GRB35 and FJ493540 for GRB68.

Phylogenic trees and evolutionary distances (Knuc) of 16S rDNA sequences were calculated using the neighbor-joining model and the maximum likelihood functions. Briefly, the sequence of the two promising PGPR strains *viz*., GRB35 and GRB68 along with other gene sequences of reference taxa retrieved from the GenBank were aligned using MUSCLE incorporated in MEGA 5 (Tamura et al. 2011). Gaps in alignment were treated as missing data and the phylogenetic trees were constructed by neighbor-joining method with Kimura two-parameter model. Bootstrapping was performed with 1000 replicates.

2.7. Green house evaluation of promising PGPR for growth promotion and disease suppression

The *in vitro* study for biocontrol showed that only five PGPR showed more than 70% inhibition of *P. myriotylum* (Table 2). Hence, only these five *viz*., GRB25 – *B. cepacia*, GRB35 – *B. amyloliquefaciens*; GRB58 – *S. marcescens*; GRB68 – *S. marcescens* and GRB91 – *P. aeruginosa* were used in the green house study. For the study, earthen pots of 20 kg capacity were filled with 15 kg sieved soil (<2 mm). The soil was a clay loam Ustic Humitropept. The initial properties of the soil are pH – 5.12; organic C – 14.2 g kg^{-1}; mineral N – 42 mg kg^{-1}; Bray P – 5.4 mg kg^{-1}; Exchangeable K – 84 mg kg^{-1}. Treatments included the promising five PGPR, one already employed ginger PGPR strain (*P. aeruginosa* – IISR 51), one bactericide *i.e.* Streptomycin sulphate applied @ 1 g L^{-1} as seed treatment, a fungicide *i.e.* Metalaxyl–Mancozeb – 1.25 g L^{-1} applied as both seed treatment and soil drench @ 1.25 g L^{-1}. Since we wanted to test the effects of the promising PGPR under natural soil conditions, the soil for this green house study was not sterilized. The treatments also included an absolute control with only soil (*i.e.* without PGPR, bactericide or fungicide). In total, there were nine treatments replicated nine times (81 pots). Two sets of such treatments combinations were made. The first set was for growth promotion study, the second for testing the biocontrol potential of selected PGPR against soft rot pathogen (*P. myriotylum*) of ginger.

Table 2
In vitro screening of shortlisted ginger rhizobacteria for antagonism against *Pythium myriotylum*.

Isolate number	Inhibition (%)[a]
GRB25 – *Burkholderia cepacia*	78.51 (62.41)
GRB 35 – *Bacillus amyloliquefaciens*	78.51 (64.81)
GRB 58 – *Serratia marcescens*	85.92 (67.99)
GRB 68 – *Serratia marcescens*	84.07 (66.45)
GRB 91 – *Pseudomonas aeruginosa*	73.93 (58.88)
Control	0.0 (4.9)
LSD ($P < 0.05$)	53

[a] Figures in the parentheses are Arc Sine transformed values

For planting, small shallow pits were made and the seed-rhizomes (20 g each) of ginger (var. Varada) with at least two sprouted buds was soaked in 1% starch solution containing the respective PGPR suspensions (~ $\times 10^7$ cfu mL^{-1}) for 1 h, shade dried for 24 h and planted @ 3 rhizomes per pot. The treated rhizomes were placed 3.5–5.0 cm deep in the pits and the soil pressed over it. The treatments also included rhizome treatment with a bactericide *viz*., Streptomycin sulphate @ 1 g L^{-1} and seed treatment+ soil drench with a fungicide *viz*., Metalaxyl–Mancozeb @ 1.25 g L^{-1}. Booster doses of each PGPR was given at 30, 60 and 90 days of planting (DAP) @ 1 L per pot (10^8 cfu mL^{-1}). All the pots were applied with major nutrients at rates equivalent to the recommended dose (RD) of NPK for ginger which is 75–50–50 kg ha^{-1} in the study site. The inorganic sources of NPK used were urea, rock phosphate (RP) and muriate of potash (MOP) respectively. RP was applied as basal dose, urea and MOP in two splits (45th and 90th day after planting (DAP)). Observation on sprouting per cent was recorded on 21st day after planting (DAP).

The second set of pots had identical treatments as described above. However, on 30 DAP, the soft rot causing pathogen (*P. myriotylum*) was challenge inoculated into all the pots. Briefly, 10 g of 5 day old mycelia of *P. myriotylum* was blended in 100 mL sterile distilled water and made up to 1000 mL and 100 mL of this mycelial suspension was inoculated into the pots. Observations on sprouting, number of tillers, rhizome yield pot^{-1}, yield $plant^{-1}$ and soft rot incidence were recorded. Rhizome rotting was assessed on a 0–4 scale: 0 = no infection; 1 = 1–25% infected area; 2 = 26–50% infected and rotten area; 3 = 51–75% rotten area; 4 = 76–100% rotten area. The per cent disease index (PDI) was calculated as follows: Disease index = Σ of all disease ratings/Total number of plant ratings × highest numerical rating × 100.

2.8. Field experiments using promising PGPR for growth promotion and disease suppression

The first field experiment with identical treatments as adopted in the green house was conducted during June 2010–February 2011 to study the effects of PGPR on growth promotion and disease suppression in field grown ginger. However, no deliberate pathogen inoculation was done; instead the experiment was conducted in sick plots. The experiment had six replications. Based on this field experiment, the two best treatments *viz*., GRB35 (*B. amyloliquefaciens*) and GRB68 (*S. marcescens*) were used for the second field experiment (June 2012–February 2013) and compared with the already employed PGPR (IISR 51 – *P. aeruginosa*), two chemical treatments *viz*., a bactericide – Streptomycin sulphate (1 g L^{-1} as seed treatment) and Metalaxyl–Mancozeb (1.25 g L^{-1} as both seed treatment and soil drench) and a control treatment where no PGPR or bactericide or fungicide were applied. This experiment also had six replications.

The field experiments were conducted at the Experimental Farm (11°35′0″ N 75°49′0″ E) of the Indian Institute of Spices Research (IISR) at Calicut, Kerala State, India. The mean annual precipitation is 4374.0 mm spread over 7 months from May to November. The dry season lasts from December to April. The site experiences tropical monsoon climate characterized by persistent high temperatures (Max – 35 °C) which normally do not go below 18 °C even in the coolest months. The soil of the study site is clay loam Ustic Humitropept. The initial properties of the soil are pH – 5.12; organic C – 14.2 g kg^{-1}; mineral N – 105 mg kg^{-1}; Bray P – 13.4 mg kg^{-1}; Exchangeable K – 164 mg kg^{-1}.

Ginger is generally grown under rainfed conditions on raised beds in Kerala State, India. For preparation of the beds, the land was cleared of weeds, the predominant ones being *Ageratum conyzoides* L., *Tridax procumbens* L., *Scoparia dulcis* L. and *Alternanthera sessilis* (L.) R. Br. Ex DC. The soil was then tilled with a tractor mounted disk harrow, puddled to a fine tilth and leveled using a soil leveler. Raised beds of size 3 × 1 × 0.30 m (l × b × h) were made and a spacing of 40 cm was allowed between the beds for drainage. Small shallow pits for planting seed rhizome were then made on the beds at a spacing of 20 × 25 cm with a plant population of 40 bed^{-1}. The seed-rhizome (20–30 g) with at least two sprouted eye buds was placed 3.5–5.0 cm deep in the pits and the soil pressed over it.

PGPR treatment was given both as seed treatment and as soil drench. In case of seed treatment, prior to sowing, the ginger rhizomes were treated with 1% starch solution containing the respective PGPR suspensions (10^{10} cfu mL^{-1}) for one hour, shade dried for 24 h and planted. In case of soil drench, the PGPR suspension (10^{8} cfu mL^{-1}) were applied at the rate of 2 L bed^{-1} at three regular intervals *viz*., 30, 60 and 90 DAP. Sprouting was recorded on 45 DAP. Disease incidence was recorded at regular intervals and PDI was calculated as described earlier. Ginger rhizome yield was recorded at harvest (240 DAP).

2.9. Statistics

The significance of treatment effects wherever applicable was determined by one-way ANOVA. All percentage values for disease incidence were arcsine square root transformed before further statistical analysis to improve the homogeneity of the variance of the data. Where the F values were significant, *post hoc* comparisons of means were made using the Least Significance Test (LSD) or Duncan's Multiple Range Test (DMRT) at the 0.05 probability level.

3. Results

A total of 100 PGPR were obtained from the rhizosphere of different varieties of ginger (Table 3). The PGPR were found to belong to 10 genera (Fig. 1) *viz*., *Pseudomonas* (33), *Serratia* (18), *Bacillus* (13), *Burkholderia* (9), *Klebsiella* (8), *Enterobacter* (6), *Arthrobacter* (5), *Micrococcus* (5) and *Curtobacterium* (1). However, two strains could not be identified. All the isolates were studied for their nutrient solubilization and growth promoting, hydrolytic enzymes and secondary metabolite production traits. However, due to the vastness of the data, we provide here only the details of the promising isolates (8 nos) with multiple PGP traits (Table 1).

3.1. Morphological and biochemical traits of the rhizobacteria isolated from ginger

All the isolates were motile rods and non spore formers (Table 4). Out of the 100 isolates, 74 were Gram-negative while 26 were Gram-positive. Besides, 41 were positive for IAA production, 41 for NH_3 production, 74 for citrate utilization, 33 for VP, 9 for methyl red test, 18 for starch hydrolysis, 33 for succinic acid production, 33 for HCN production and 46 for oxidase. With regard to production

Table 3
Details of ginger rhizobacteria (GRB) isolated from various locations of South India.

Isolate no	Place of collection	Ginger variety	Genera and total no of isolates
GRB 1-12	Peruvannamuzhi, Kerala State, India	Varada Rejatha Mahima Maran Himachal Suprabha	*Arthrobacter*, *Micrococcus*, *Pseudomonas*, *Burkholderia*, *Serratia*, *Klebsiella* (12)
GRB 13-48	Peruvannamuzhi, Kerala State, India	Varada	*Bacillus*, *Arthrobacter*, *Micrococcus*, *Pseudomonas*, *Enterobacter*, *Burkholderia*, *Serratia*, *Klebsiella* (36)
GRB 49-69	Kodagu, Karnataka State, India	Varada Rejatha Mahima	*Bacillus*, *Curtobacterium*, *Pseudomonas*, *Enterobacter*, *Burkholderia*, *Serratia*, *Klebsiella* (21)
GRB 70-100	Ambalavayal, Kerala State, India	PGS V2E52 Dhanja Mizoram RARS-1 Mahima Rio-De-Janeiro	*Bacillus*, *Arthrobacter*, *Pseudomonas*, *Enterobacter*, *Burkholderia*, *Serratia*, *Klebsiella* (31)

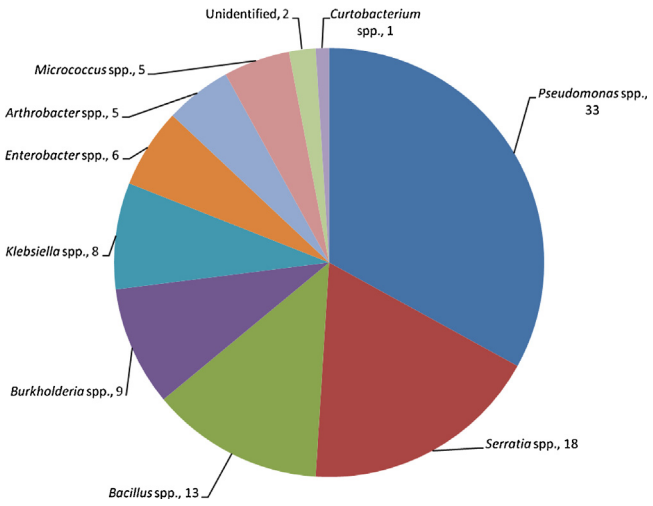

Fig. 1. Major genera of rhizobacteria isolated from different varieties of ginger.

of hydrolytic enzymes, 27 isolates were positive for protease, 14 for cellulase, 11 for pectinase and 12 for amylase. Nevertheless, all the isolates were positive for catalase production, but negative for indole production. When tested for their survivability at varying concentrations (2%, 5%, 7% and 10%) of NaCl, the results revealed that most of the isolates were able to survive at the lower two concentrations, while all the 18 isolates of *Serratia* spp. could survive at all concentrations. Likewise, when tested for survival at varying temperatures (4 °C, 28 °C, 37 °C, 40 °C, 45 °C and 55 °C), it was observed that *Pseudomonas* spp., *Burkholderia* spp. and *Klebsiella* spp. could tolerate temperatures between 28 and 40 °C, *Serratia* spp. and *Enterobacter* spp. between 4 and 45 °C, *Bacillus* spp. and *Arthrobacter* spp. between 28 and 45 °C and *Micrococcus* spp. between 28 and 55 °C. With regard to production of hydrolytic

Table 4
Morphological and biochemical traits of the rhizobacteria isolated from ginger.

Rhizobacterial isolates[a]	Morphological and biochemical traits	Genera and number of isolates
GRB3, GRB4, GRB15, GRB20, GRB21, GRB22, GRB23, GRB31, GRB32, GRB33, GRB38, GRB39, GRB40, GRB41, GRB43, GRB45, GRB46, GRB47, GRB51, GRB53, GRB54, GRB73, GRB75, GRB77, GRB80, GRB83, GRB88, GRB90, GRB 91, GRB93, GRB95, GRB97, GRB99	G-, short rods, motile, Non spore formers, indole (−), methyl red (−), Voges-Proskauer (−), citrate (+), catalase (+), oxidase (+), succinic acid (+), casein hydrolysis (+), starch hydrolysis (−), smmonia production (+), HCN production (+), growth in NaCl 2% (+), 5% (+), 7% (−), 10% (−), growth at 4 °C (−), 28 °C (+), 37 °C (+), 40 °c (+), 45 °C (−), 55 °C (−)	*Pseudomonas* spp. (33)
GRB 7, GRB 9, GRB 13, GRB 24, GRB34, GRB 42, GRB 49, GRB 52, GRB37, GRB58, GRB 59, GRB 60, GRB 61, GRB 62, GRB 64, GRB65, GRB 66, GRB68	G-, rods, motile, Non spore formers, indole (−), methyl red (−), Voges-Proskauer (+), citrate (+), catalase (+), oxidase (−), succinic acid (−), casein hydrolysis (+), starch hydrolysis (−), ammonia production (−), HCN production (+), growth in NaCl 2% (+), 5% (+), 7% (+), 10% (+), growth at 4 °C (+), 28 °C (+), 37 °C (+), 40 °c (+), 45 °C (+), 55 °C (−)	*Serratia* spp. (18)
GRB16, GRB55, GRB70, GRB71, GRB89, GRB96	G-, rods, motile, non spore formers, indole (−), methyl red (+), Voges-Proskauer (+), citrate (+), catalase (+), oxidase (−), succinic acid (−), casein hydrolysis (+), starch hydrolysis (−), ammonia production (−), HCN production (−), growth in NaCl 2% (+), 5% (+), 7% (+), 10% (+), growth at 4 °C (+), 28 °C (+), 37 °C (+), 40 °c (+), 45 °C (+), 55 °C (−)	*Enterobacter* spp. (6)
GRB 10, GRB36, GRB50, GRB56, GRB 74, GRB82, GRB94, GRB98	G-, short rods, motile, non spore formers, indole (−), methyl red (−), Voges-Proskauer (−), citrate (+), catalase (+), oxidase (+), Succinic acid (−), casein hydrolysis (+), starch hydrolysis (−), ammonia production (+), HCN production (−), growth in NaCl 2% (+), 5% (+), 7% (−), 10% (−), growth at 4 °C (−), 28 °C (+), 37 °C (+), 40 °c (+), 45 °C (−), 55 °C (−)	*Klebsiella* spp. (8)
GRB6, GRB17, GRB25, GRB28, GRB63, GRB79, GRB84, GRB86, GRB92	G-, short rods, motile, non spore formers, indole (−), methyl red (−), Voges-Proskauer (+), citrate (+), catalase (+), oxidase (−), succinic acid (−), casein hydrolysis (+), starch hydrolysis (−), ammonia production (±), HCN production (−), growth in NaCl 2% (+), 5% (+), 7% (−), 10% (−), growth at 4 °C (−), 28 °C (+), 37 °C (+), 40 °C (+), 45 °C (−), 55 °C (−)	*Burkholderia* spp. (9)
GRB8, GRB18, GRB19, GRB27, GRB 28, GRB 29, GRB35, GRB48, GRB69, GRB72, GRB81, GRB85, GRB87	G+, long rods, motile, non spore formers, indole (−), methyl red (−), Voges-Proskauer (−), citrate (−), catalase (+), oxidase (−), succinic acid (−), casein hydrolysis (+), starch hydrolysis (+), ammonia production (±), HCN production (+), growth in NaCl 2% (+), 5% (+), 7% (+), 10% (−), growth at 4 °C (−), 28 °C (+), 37 °C (+), 40 °C (+), 45 °C (+), 55 °C (+)	*Bacillus* spp. (13)
GRB1, GRB30, GRB76, GRB 78, GRB 100	G+, long rods, motile, non spore formers, indole (−), methyl red (−), Voges-Proskauer (−), citrate (−), catalase (+), oxidase (−), succinic acid (−), casein hydrolysis (+), starch hydrolysis (+), ammonia production (±), HCN production (−) growth in NaCl 2% (+), 5% (−), 7% (−), 10% (−), growth at 4 °C (−), 28 °C (+), 37 °C (+), 40 °c (+), 45 °C (+), 55 °C (−)	*Arthrobacter* spp. (5)
GRB2, GRB11, GRB12, GRB14, GRB26	G+, coccus, non motile, non spore formers, indole (−), methyl red (−), Voges-Proskauer (−), citrate (−), catalase (+), oxidase (+), succinic acid (−), casein hydrolysis (−), starch hydrolysis (−), ammonia production (±), HCN production (−), growth in NaCl 2% (+), 5% (+), 7% (+), 10% (−), growth at 4 °C (−), 28 °C (+), 37 °C (+), 40 °C (+), 45 °C (+), 55 °C (+)	*Micrococcus* spp. (5)
GRB57	G+, short rods, motile, non spore formers, indole (−), methyl red (−), Voges-Proskauer (−), citrate (−), catalase (+), oxidase (−), succinic acid (−), casein hydrolysis (+), starch hydrolysis (−), ammonia production (±), HCN production (−), growth in NaCl 2% (+), 5% (+), 7% (−), 10% (−), growth at 4 °C (−), 28 °C (+), 37 °C (+), 40 °C (+), 45 °C (−), 55 °C (−)	*Curtobacterium* spp. (1)

[a] GRB – Ginger rhizobacteria; two isolates (GRB5 and GRB44) could not be identified.

enzymes, out of the 100 isolates, 27 isolates were positive for protease, 14 for cellulase, 11 for pectinase and 12 for α-amylase.

3.2. Nutrient solubilization traits of the isolates

Out of the 100 isolates, 39 could solubilize P while a vast majority (79) produced NH_3. Among these, five isolates *viz*., GRB2 (*Micrococcus* spp.), GRB25 (*B. cepaceae*), GRB36 (*Klebsiella sp.*), GRB38 (*S. marcescens*), GRB70 and 71 (*Enterobacter sp.*) showed the maximum P solubilization and NH_3 production capacity. In case of Zn solubilization, only 9 of the 100 tested positive. Of these, 2 isolates *viz*., GRB 25 (*B. cepaceae*) and GRB38 (*S. marcescens*) showed relatively greater Zn solubilization capacity. Of the 100 isolates, 28 were found to solubilize K and 9 could solubilize Si. Of these, GRB 25 and 70 showed relatively greater K solubilization capacity, while GRB 36 and 38 showed relatively greater Si solubilization capacity.

3.3. Antifungal activity of the isolates

All the isolates were screened against *P. myriotylum* Drechs., causing soft rot in ginger. The results (Table 2) revealed that 5 of the 100 isolates (GRB25 – *B. cepacia*; GRB35 – *B. amyloliquefaciens*; GRB58 – *S. marcescens*; GRB68 – *S. marcescens* (Fig. 2) and GRB91 – *P. aeruginosa*) exhibited >70% inhibition of *P. myriotylum*.

3.4. Effect of shortlisted isolates on ginger sprouting and disease suppression in the green house

The rhizomes treated with GRB35 (*B. amyloliquefaciens*) and GRB68 (*S. marcescens)* registered 96.3% sprouting (germination), while those treated with GRB58, IISR51, Streptomycin sulphate and Metalaxyl–Mancozeb registered 88.9% sprouting (Table 5). The shortlisted isolates were also tested for their biocontrol

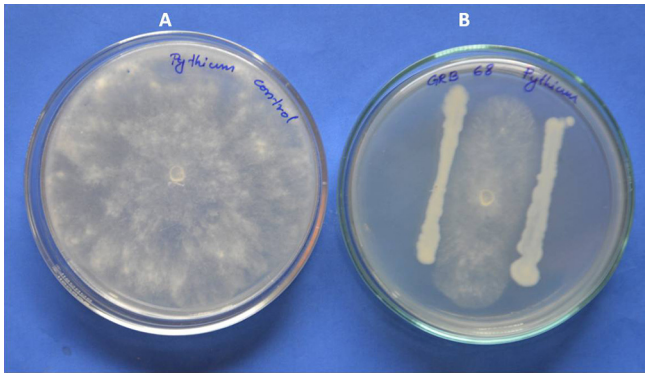

Fig. 2. Inhibition of soft rot pathogen *Pythium myriotylum* by the PGPR strain GRB68 (*S. marcescens*); (A) Control (pathogen alone); (B) GRB68 dual cultured with *P. myriotylum*.

Table 5
Effect of PGPR and chemical treatments on sprouting (%), soft rot incidence (%) and rhizome yield (g pot^{-1}) of ginger in the green house.

Treatment[a]	Sprouting[b] (%)	Soft rot incidence[b] (%)	Yield (g pot^{-1})
GRB25	81.3 (66.0) a[c]	96.3 (85.0) a	190.0 b
GRB35	96.3 (74.6) a	48.1 (42.6) bc	365.6 a
GRB58	88.9 (70.4) a	92.6 (81.2) a	334.4 a
GRB68	96.5 (75.1) a	57.4 (54.3) bc	384.4 a
GRB91	85.2 (67.5) a	44.4 (41.4) bc	186.7 b
IISR51	88.6 (70.0) a	62.9 (58.5) bc	186.1 b
Streptomycin sulphate	88.5 (70.0) a	74.1 (69.5) ab	152.8 c
Metalaxyl–Mancozeb	88.9 (70.3) a	51.8 (47.6) bc	152.8 c
Control	66.6 (53.2) b	100 (88.9) a	134.4 c

[a] GRB25 – *Burkholderia cepacia*; GRB35 – *Bacillus amyloliquefaciens*; GRB58 – *Serratia marcescens*; GRB68 – *Serratia marcescens*; GRB91 – *Pseudomonas aeruginosa*; IISR51 – *Pseudomonas aeruginosa*; Streptomycin sulphate @ 1.0 g L^{-1}; T8 – Metalaxyl–Mancozeb @ 1.25 g L^{-1} as seed treatment + soil drench; T9 – control.
[b] Figures in parentheses are Arc Sine transformed values.
[c] In a column, means followed by the same letter are not significantly different at $P < 0.05$.

Table 6
Effect of PGPR and chemical treatments on sprouting (%), soft rot incidence (%) and rhizome yield (g pot^{-1}) of ginger in the field.

Treatment[a]	Sprouting[b] (%)	Soft rot[b] (%)	Rhizome yield (kg^3 m^{-2})
GRB25	76.2 (60.9) bc[c]	55.6 (48.2) a	2.0 b
GRB35	92.2 (73.9) a	38.2 (38.2) cd	3.8 a
GRB58	78.4(62.4) bc	45.8 (42.6) bc	3.2 a
GRB68	93.0 (74.7) a	26.6 (31.1) e	4.1 a
GRB91	89.5 (71.0) a	38.6 (38.4) cd	3.4 a
IISR51	76.5 (61.0) bc	50.4 (45.2) ab	2.0 b
Streptomycin sulphate	85.5 (67.6) ab	44.2 (41.7) bc	3.0 ab
Metalaxyl–Mancozeb	86.2 (68.3) ab	41.3 (41.7) c	2.0 b
Control	84.7 (67.1) ab	62.8 (52.4) a	1.0 bc

[a] GRB25 – *Burkholderia cepacia*; GRB35 – *Bacillus amyloliquefaciens*; GRB58 – *Serratia marcescens*; GRB68 – *Serratia* marcescens; GRB91 – *Pseudomonas aeruginosa*; IISR51 – *Pseudomonas aeruginosa*; Streptomycin sulphate @ 1.0 g L^{-1}; T8 – Metalaxyl–Mancozeb @ 1.25 g L^{-1} as seed treatment + soil drench; T9 – control.
[b] Figures in parentheses are Arc Sine transformed values.
[c] In a column, means followed by the same letter are not significantly different at $P < 0.05$.

potential against soft rot (*P. myriotylum*). Least incidence (44.4%) of soft rot was registered in the treatment involving GRB91 (*P. aeruginosa*) closely followed by GRB35 (*B. amyloliquefaciens*) with 48.1% incidence and GRB68 (*S. marcescens*) with 57.4% incidence. However, they were at par with the treatment involving Metalaxyl–Mancozeb, which registered 51.8% incidence. The rhizome yield was markedly higher in treatments with GRB35 (365.6 g pot^{-1}), GRB58 (334.4 g pot^{-1}) and GRB68 (384.4 g pot^{-1}) compared to IISR51 and the two chemical treatments.

3.5. Effect of shortlisted isolates on ginger sprouting and disease suppression in the field

In the first field experiment (Table 6) with similar treatments conducted in sick plots, GRB68 (*S. marcescens*) and GRB35 (*B. amyloliquefaciens*) registered the greatest sprouting (93.0 and 92.2%, respectively) but were on par with chemical treatments (Streptomycin sulphate and Metalaxyl–Mancozeb). Similarly, GRB68 (*S. marcescens)* and GRB35 *(B. amyloliquefaciens*) registered lowest soft rot incidence (26.6 and 38.2%, respectively) and greater rhizome yield (4.1 and 3.8 kg^3 m^{-2}, respectively). From the green house experiment and field experiment, the results revealed that only two PGPR *viz*., GRB35 (*B. amyloliquefaciens*) and GRB68 (*S. marcescens*) were the most promising. Hence, in the second field experiment, these two PGPR were compared with an already popular PGPR for ginger (IISR51 – *P aeruginosa*), a bactericide (Streptomycin sulphate) and a fungicide (Metalaxyl–Mancozeb). The results (Table 7) revealed that the treatments with GRB35 and GRB68 once again registered markedly lower soft rot incidence (5.2 and 7.3%, respectively), greater sprouting (80%) and higher yield (5.0 and 4.3 kg^3 m^{-2}, respectively) compared to the other treatments. In fact, compared to Streptomycin sulphate treatment, the yield increase in GRB35 and GRB68 treatments was 117% and 87%, respectively and compared to Metalaxyl–Mancozeb the yield increase was slightly lower at 31% and 13%, respectively.

4. Discussion

In this study, about 100 PGPR were isolated from ginger, majority of which belonged to the genera *Pseudomonas*, followed by *Serratia*, *Bacillus*, and *Burkholderia.* This result is comparable to numerous studies that have reported that the most representative genera are *Pseudomonas*, *Enterobacter*, *Clostridium*, *Arthrobacter*, *Achromobacter*, *Micrococcus*, *Flavobacterium*, *Azospirillum*, *Azotobacter* and *Bacillus*, with the latter being the most common group of bacteria isolated from soil and other environments (Felici et al. 2008; Forchetti et al. 2007; Swain and Ray 2009; Vessey 2003).

It has been reported that the commercial application of these bacteria for biocontrol has been hampered in large part because of their inconsistent performance between field locations due to variations in climate and soil (Ownley et al. 2003). Hence, we screened the 100 PGPR for an array of biochemical and nutrient mobilization traits in order to select the ones that showed promise with regard to biocontrol and growth promotion in ginger. The results revealed that some of the PGPR exhibited multiple PGP traits *in vitro*. Such multiple modes of action have been reported to be the prime reasons for the plant growth promotion and disease suppressing ability of PGPR (Antoun and Prévost 2005; Bashan and de-Bashan 2010). Similar to our study, a single PGPR has often been found to reveal multiple modes of action including biological control (Antoun and Prévost 2005). Besides, a large number of PGPR belonging to different bacterial classes and genera with multifunctional traits have been described (Rodríguez-Díaz et al. 2008).

The experiment on *in vitro* screening of rhizobacteria for antagonism *against P. myriotylum* indicated that 5 isolates (GRB25 – *B. cepacia*; GRB35 – *B. amyloliquefaciens*; GRB58 – *S. marcescens*;

Table 7
Effect of promising PGPR (GRB35 and GRB68) on sprouting, soft rot incidence and rhizome yield of ginger in the field (mean of six replications).

Treatment[a,b]	Sprouting (%)	Soft rot (%)	Yield ($kg^3\ m^{-2}$)
GRB35	80.0 a	5.2 d	5.0 a
GRB68	80.0 a	7.3 d	4.3 ab
IISR51	74.5 b	11.6 c	3.0 bc
Streptomycin sulphate	73.0 b	18.5 b	2.3 c
Metalaxyl–Mancozeb	73.0 b	14.0 c	3.8 b
Control	73.0 b	25.1 a	2.2 c

[a] GRB35 – *Bacillus amyloliquefaciens*; GRB68 – *Serratia marcescens*; IISR51 – *Pseudomonas aeruginosa*; Streptomycin sulphate applied @ $1\ g\ L^{-1}$ as seed treatment; Metalaxyl–Mancozeb applied @ $1.25\ g\ L^{-1}$ as both seed treatment and soil drench.
[b] In a column, means followed by the same letter are not significantly different at $P < 0.05$.

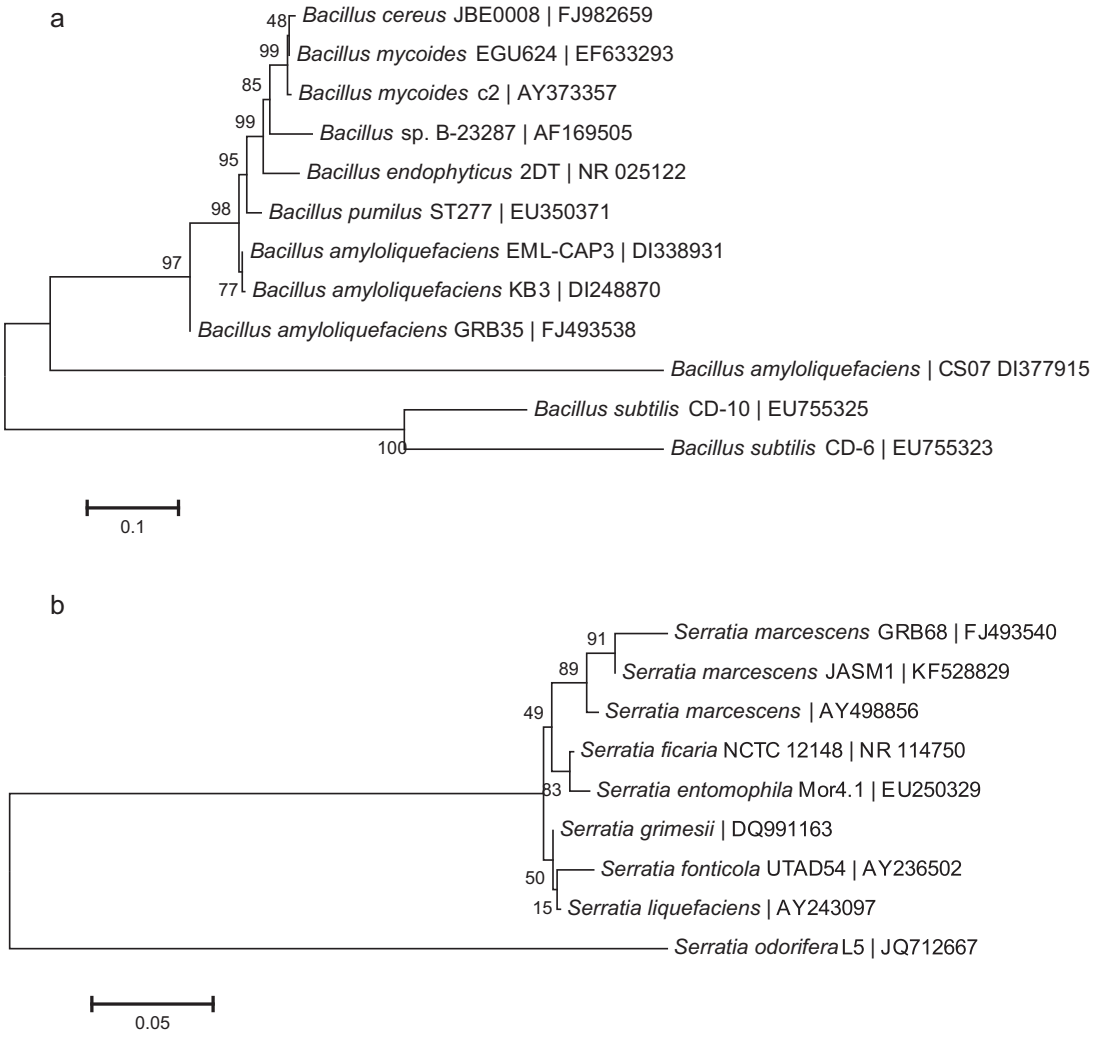

Fig. 3. (a, b) Neighbor-joining tree based on analysis of partial 16S rDNA nucleotide sequences of *Bacillus* spp and *Serratia* spp. Numbers above or below the nodes indicate bootstrap values generated after 1000 replications. Bacteria species and sequences obtained from GenBank are shown with their accession numbers in the figure. The tree is drawn to scale, with branch lengths in the same units as those of the evolutionary distances used to infer the phylogenetic tree. The evolutionary distances were computed using the Kimura 2-parameter method and are in the units of the number of base substitutions per site.

GRB68 – *S. marcescens* and GRB91 – *P. aeruginosa*) exhibited >70% inhibition of *P. myriotylum*. Such severe antifungal effects *in vitro* can be attributed to the production of a variety of antimicrobial compounds that cause cytolysis, leakage of potassium ions, disruption of the structural integrity of membranes, inhibition of mycelial growth, inhibition of spore germination and protein biosynthesis (Quan et al. 2010; Yuan et al. 2012).

The promising 5 PGPR were then tested in the greenhouse for their growth promotion and disease suppressing ability compared to Streptomycin sulphate and Metalaxyl–Mancozeb. The soil used in the study was not sterilized because an important factor to consider during screening of new PGPR is their activity in the soil type/conditions wherein they are expected to be used (Rana et al. 2011; Ross et al. 2000). Nevertheless, the results from green house and both field experiments suggested that the PGPR differed in their effects on sprouting, disease incidence and yield in ginger and of these, two PGPR *viz*., GRB35 (*B. amyloliquefaciens*) and GRB68 (*S. marcescens*) performed better than even the chemical treatments

(Streptomycin sulphate and Metalaxyl–Mancozeb). Earlier reports have also indicated that the PGP and disease suppressing ability of PGPR is due to multiple modes of action (Antoun and Prévost 2005; Bashan and de-Bashan 2010) and in most cases, a single PGPR will often reveal multiple modes of action including biological control (Vessey 2003). Similar to our results on ginger, several studies on growth promotion by PGPR in other crops like maize (Egamberdiyeva 2007), common bean (Egamberdieva 2011), ground nut (Goswami et al. 2014), tomato (Almaghrabi et al. 2013) and *Bt*-cotton (Kumar and Gera 2014) have been reported.

The results further suggested that both GRB35 (*B. amyloliquefaciens*) and GRB68 (*S. marcescens*) markedly reduced soft rot incidence cause by *P. myriotylum* and even outperformed chemical treatments like Streptomycin sulphate and Metalaxyl–Mancozeb. Such inhibition by the two PGPR can be attributed to production of secondary metabolites (such as HCN, NH_3 *etc.*) that inhibit phytopathogens or by competing for colonization sites, nutrients, *etc.* (Bhattacharyya and Jha 2012; Martínez-Viveros et al. 2010). Besides, the marked antifungal activity of *B. amyloliquefaciens* as observed in our study can be attributed to its ability to produce several bacillomycin- and macrolactin-type antibiotics (Yuan et al. 2012) and it has been found that the cyclic lipopeptides like surfactin, iturin, and fengycin produced by *B. amyloliquefaciens* have potent antimicrobial activity (Fernandes et al. 2007). Similar to our studies, *B. amyloliquefaciens* has been found to suppress wilt (*Fusarium oxysporum*) in banana and cucumber (Xu et al. 2014; Yuan et al. 2013). Similarly, *S. marcescens* has the ability to produce chitinolytic enzymes and antibiotics and has been found to suppress damping-off, caused by *Rhizoctonia solani* and Fusarium wilt caused by *F. oxysporum* (Someya et al. 2000) and gray mold in cyclamen cause by *Botrytis cinerea* (Someya et al. 2001). Based on partial 16S rDNA analysis, GRB35 was found to be closely related to other PGPR strains like *Bacillus pumilus* ST277 (EU350371) and *B. amyloliquefaciens* EML-CAP3 (DI338931). Similarly, GRB68 was closely related to PGPR like *S. marcescens* JASM1 (KF528829) (Fig. 3a and b).

5. Conclusions

This study underlines the importance of isolating and screening PGPR for multiple PGP and biocontrol traits and evaluating the promising strains through green house and field experiments in ginger. Such comprehensive screening followed by field testing helps in identifying rhizobacterial strains adaptable to diverse environment and soil conditions. In this study, based on *in vitro*, green house and field experiments, two PGPR *viz*., GRB35 – *B. amyloliquefaciens* and GRB68 – *S. marcescens* (out of the 100 PGPR originally isolated from ginger) markedly enhanced sprouting, suppressed soft rot incidence and increased rhizome yield in ginger compared to chemical treatments like Streptomycin sulphate and Metalaxyl–Macozeb. It was, therefore, apparent that both these PGPR hold great promise as a viable alternative to chemical inputs and can be integrated into appropriate nutrient management and disease management schedules for ginger. While this study reaffirms the potential of PGPR like *B. amyloliquefaciens* and *S. marcescens* for sustainable ginger cultivation, we, however, advise caution while deploying *S. marcescens* since it has been implicated to be an opportunistic human pathogen (Mahlen 2011).

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