Biological Control 72 (2014) 9–16

Contents lists available at ScienceDirect

Biological Control

journal homepage: www.elsevier.com/locate/ybcon

Diversity of cultivable rhizobacteria across tomato growing regions of Karnataka

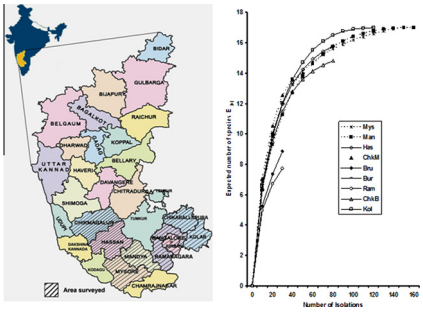
P. Hariprasad [a,*], G. Venkateswaran [b], S.R. Niranjana [c]

[a] Centre for Rural Development and Technology, Indian Institute of Technology Delhi, Hauz Khas, New Delhi 110 016, India
[b] Food Microbiology Department, Central Food Technological Research Institute, Mysore 570 020, Karnataka, India
[c] Department of Studies in Biotechnology, University of Mysore, Manasagangotri, Mysore 570 006, Karnataka, India

HIGHLIGHTS

- Rhizosphere of tomato was predominantly colonized by *Bacillus* and *Pseudomonas*.
- Analysis revealed that most rhizobacteria endowed with more than one PGPR trait.
- IAA and P solubilization are two dominant traits exhibited by rhizobacteria.
- Rhizobacteria showed a varied levels of plant growth promotion.
- Rhizobacteria offered protection against fungal origin foliar and root pathogens.

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 19 November 2013
Accepted 30 January 2014
Available online 8 February 2014

Keywords:
Rhizobacteria
Diversity
Indole acetic acid
Plant growth promotion
Fusarium wilt
Early blight

ABSTRACT

Seven hundred and fifty-two rhizobacteria were isolated from 186 rhizosphere soil samples collected across tomato growing regions of Karnataka. Among them, 26% strains were Gram positive and other 74% were Gram negative and dominant being *Bacillus* and *Pseudomonas*. Sampling of different locations showed variation in species richness and diversity indices. Similarity matrix computed with Jaccard's coefficient and principle coordinate analysis to correlate bacterial diversity revealed that rhizobacterial genera of Mysore, Mandya and Kolar soil samples were very closely related and rarefaction curve analysis indicated that these soil samples also harbored higher number of rhizobacteria which included all the genera studied. PGPR trait analysis revealed that most of the rhizobacteria were endowed with more than one beneficial trait which may act individually or simultaneously, and indole acetic acid production and phosphate solubilization are the two predominant traits exhibited by these rhizobacteria. Rhizobacterial isolates also showed a varied level of plant growth promotion traits and offered protection against fungal origin foliar and root pathogens. Among the nine regions studied, Mysore, Mandya and Kolar regions recorded higher percentage of promising PGPRs in comparison with other regions studied of Karnataka.

1. Introduction

Microorganisms in soil are critical to maintain soil functions in both natural and managed agricultural soils because of their involvement in soil structure formation; decomposition of organic matter; toxin removal; and for the operation of carbon, nitrogen, phosphorus, sulphur and such other cycles (Elsas et al., 2006). Rhizosphere is the volume of soil surrounding roots, which is influenced chemically, physically and biologically by the plant root and is a highly favorable habitat for the proliferation of microorganisms and exerts a potential impact on plant health and soil fertility

* Corresponding author.
E-mail address: phimprovement@rediffmail.com (P. Hariprasad).

http://dx.doi.org/10.1016/j.biocontrol.2014.01.012
1049-9644/

(Sorensen, 1997). Rhizodeposition through plant root exudates plays a major role in defining resident microflora, which differs from that in bulk soil (Lynch, 1990; Mittal and Johri, 2007; Micallef et al., 2009; Monterio et al., 2009). An important group of rhizobacterial community that exerts beneficial effects on plant growth upon root colonization are termed as plant growth promoting rhizobacteria (PGPR) (Kloepper and Schroth, 1978). Large numbers of rhizobacteria from diverse plant species have been reported as PGPR which include, *Aeromonas*, *Agrobacterium*, *Alcaligens*, *Azoarcus*, *Azospirillum*, *Azotobacter*, *Arthrobacter*, *Bacillus*, *Cellulomonas*, *Clostridium*, *Enterobacter*, *Erwinia*, *Flavobacterium*, *Gluconacetobacter*, *Klebsiella*, *Microbacterium*, *Micromonospora*, *Panibacillus*, *Pseudomonas*, *Rhizobia*, *Serratia*, *Streptomyces*, *Xanthomonas*, etc. have been identified as PGPR, while the search for additional strains is still continued. However, information regarding diversity and distribution of PGPR and their characters involved in plant growth promotion and disease suppression in tomato is limited.

Application of PGPR in agriculture improves plant performance under various kinds of stress environments and consequently, enhance yield by exerting various beneficial traits, also reduces the need for chemical fertilizers and pesticides, and contribute for sustainable agricultural production. Several mechanisms have been postulated to explain how PGPR stimulate plant growth which includes (i) the ability to produce or change the concentration of the plant hormones, indole acetic acid, gibberellic acid, cytokinins, ethylene and volatiles (ii) asymbiotic nitrogen fixation (iii) antagonism against phytopathogenic microorganisms by the production of siderophore, antibiotics, Chitinase, β-1,3-glucanase and cyanide and (iv) solubilization of mineral phosphate and other nutrients. Induced systemic resistance (ISR) is one of the most studied mechanism through which the rhizobacteria suppresses the infection and disease development by elevating the host resistance mechanism against wide range of phytopathogens (Van Loon et al., 1998). Similarly, Induced systemic tolerance (IST) has been proposed for PGPR induced physiological and biochemical changes in plants that result in enhanced tolerance to abiotic stress (Yang et al., 2009). PGPR mediated improvement of plant health is through expressing one or more traits individually or simultaneously which depends on various biotic and abiotic variables at rhizosphere (Glick et al., 1999; Rana et al., 2011; Hariprasad et al., 2011, 2013).

Studying the diversity of rhizobacteria in a particular crop of specific geographical region will be an important milestone as screening will results in potential rhizobacteria which can successfully used as PGPR. Because, the probability of root colonization by native rhizobacteria is high in comparison with the rhizobacteria isolated from other regions or other crops. Hence, the present study was directed towards assessment of community composition of rhizobacteria in the rhizosphere of tomato and also their diverse mechanism of plant growth promotion and disease suppression across tomato growing regions of Karnataka. Our interest is to develop efficient biofertilizer and biopesticides using native rhizobacteria of tomato plants with multiple PGPR traits.

2. Materials and methods

2.1. Field survey – soil sample collection

One hundred and eighty-six rhizosphere soil samples were collected from tomato plants cultivated in various agro climatic regions of Karnataka during June, 2008 – May, 2009. Rhizosphere soil samples were collected from healthy field-grown plants which were at flowering/pre-flowering stage. Five plants, each with root and rhizospheric soil, were harvested from five different quadrates in each field and samples from each field were pooled together to make a composite soil sample. Rhizosphere soil samples were placed in polybags, transported to the laboratory, stored at 4 °C and processed within 48 h of collection.

2.2. Microorganisms

In order to isolate rhizobacteria from the rhizosphere soil sample, 10 g of soil were suspended in 90 ml of saline (0.85% NaCl), shaken for 30 min, and the suspension was then serially diluted (8-fold) and plated onto Luria bertani (LB) agar medium in Petri dishes which were incubated at 37 ± 2 °C. At the end of 36–48 h of incubation, individual colonies which differed in their colony morphology (colony character) were transferred to LB agar slants. To avoid other saprophytic bacteria from bulk soil, those bacterial colonies were only isolated whose population was more than 10% of total bacterial population of any particular rhizospheric soil sample. All the rhizobacterial isolates were maintained on LB agar slants at 4 °C for short term storage and for long term storage, the bacteria were stored in 40% glycerol at −80 °C (Terezia et al., 2008).

Plant pathogenic fungi, *Fusarium oxysporum* f. sp. *lycopersici* (UOM/Bt-Fol-012) and *Alternaria solani* (UOM/Bt-As-007) the causal agents of *Fusarium* wilt and Early blight, respectively in tomato were collected from fungal culture collection of Department of Biotechnology, University of Mysore, India. Both fungus were maintained on potato dextrose agar (PDA) slants at 4 °C till further use. Inoculum was prepared by culturing the fungus on PDA medium for 7 days in Petri plates. Conidial suspension was prepared by pouring 10 ml of sterile distilled water in each Petri plate. The concentration of microconidia for *F. oxysporum* was adjusted to 1000 conidia/ml. Similarly for *A. solani*, the conidial concentration was adjusted to 5×10^4 conidia/ml and used for greenhouse experiments.

2.3. Identification and characterization of rhizobacteria for PGPR traits

The identities of the rhizobacterial isolates up to genus level were established by studying their staining and biochemical properties, according to Bergey's Manual of Systemic Bacteriology (Krieg and Holt, 1984) which includes, Gram's staining, Acid Fast staining, Negative staining, Lactose Fermentation, Indole, Methyl Red, Voges-Proskauer, Citrate Utilization, Urease, Nitrate Reduction, Oxidase, Catalase, Hydrogen Sulphide Production, Aerobic and Anerobic growth Test. For selected isolates, the 16S rRNA gene was amplified using primers (27F, 50-AGAGTTTGATCCTGGCTCAG-30 and 1494R 50-TTCGATACGGGCAGGCTAGAGT-30) as previously described (Hariprasad et al., 2013) and the resulting amplicons were sequenced using different internal primers (Lane, 1991). The 16S rRNA gene sequences were then subjected to BLAST sequence similarity search (Altschul et al., 1990) to identify up to species level. Further rhizobacterial isolates were analyzed for their various PGPR traits. Briefly,

(i) Root colonization bioassay. This was carried out following standard procedures of Silva et al. (2003). The bacterial isolates that colonized roots after repeated tests were selected for further studies.
(ii) 1-Amino cyclopropane-1-carboxylate (ACC) deaminase activity. Production of ACC deaminase was determined as described by Penrose and Glick (2003) by the previously described defined medium, except the C source were sucrose (5.0 g l^{-1}), mannitol (5.0 g l^{-1}), and sodium lactate (0.5 ml of a 5.4 M solution) and the N source was ACC (5.0 g l^{-1}).
(iii) Siderophore. Siderophore production was determined as described by Schwyn and Neilands (1987) using blue indicator dye, chrome azurol S (CAS). Bacterial isolates exhibiting an orange halo after 5 days of incubation at 28 ± 2 °C were considered positive for the production of siderophores.