



Original Research Article

Structural and Functional Diversity of Rhizobacterial Strains Isolated from Rhizospheric Zone of Different Plants of Sholapur- Maharashtra Region, India

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ABSTRACT

In the present study a research was carried out to examine the structural and functional diversity of rhizobacteria associated with plant roots cultivated in agro climatic zone of district Sholapur, Maharashtra, India. Five different plants were selected from the region in the month of October. Rhizobacterial strains were isolated from plants such as Coriander, Onion, Potato, Tomato and Turmeric on nutrient agar plate by spread plate technique. Colonies showing distinct morphology were selected, a total 27 colonies were maintained on nutrient agar slants for further studies, 7 from coriander, 5 from onion, 6 from potato, 4 from tomato and 5 from turmeric rhizospheric zones. All organisms were found to be fast growers with generation time more $3.5 \pm 0.3 \text{ h}^{-1}$. Functional diversity was assessed on the basis of their efficiency in controlling the growth of phytopathogen *Sclerotium rolsfii* and its plant growth promotory rhizobacterial traits (PGPR) such as production of indole acetic acid, siderophore, hydrocyanic acid, phosphate and chitin solubilizations. A marked variation in terms of structural and functional diversity was recorded. Structural diversity was recorded highest from Coriander rhizosphere. The findings were supported by its higher Simpsons indices value. On the other hand, when PGPR traits were observed it was observed that bacterial isolates from Coriander and Turmeric were recorded as highest producer of IAA, siderophore, HCN and solubilizers of chitin and phosphate as compared to bacterial isolates of other plants. Similarly highest biocontrol efficiency in controlling growth of phytopathogen of *S. rolsfii* was found in Coriander and Turmeric rhizospheric isolates specifically cor-4 and tur-3.

Keywords

Diversity,
Plant growth
promotory
rhizobacterial
traits,
Phytopathogen,
*Sclerotium
rolsfii*

Introduction

Soil is defined as one of the most valuable and dynamic non-renewable resources of ecosystem. Due to its chemical, physical and biological components dynamic equilibrium is being maintained (Buscot, 2005). A large

portion of the earth's biodiversity is being represented by soil biota. The biota (organisms) carries out an indescribable range of processes for maintaining soil resource qualities. The biota also many

times disturbs its stability and generates biodiversity among them. Biodiversity is the one of the most imperative feature of environmental maintenance and most desirable task in production of agriculture (Mittal and Johri, 2007). In the system as a cause of diversity, change in functional and genetic activity of diverse microorganisms that imparts a crucial affect on soil functions (Nannipieri *et al.*, 2003) and indirectly effect plant health and productivity. Communities of soil interact with plant roots and constituents of soil at the root soil boundary (Attitalla *et al.*, 2011), that is defined as rhizosphere. It is the zone that is rich in nutrients as range of organic compounds released from the roots by a process called exudation, deposition and secretion (Shukla *et al.*, 2011). The colonization pattern of rhizobacteria is affected by plant species, plant type, plant age and exudates of root (Mahaffee and Kloepper, 1997). The bacterial species that colonize the rhizospheric zone are known as rhizobacteria (Yasmin *et al.*, 2007; Zaidi *et al.*, 2009). The rhizobacteria have been well known from many decades for their importance in enhancing the nutrient level of the soil that indirectly promotes the growth of the plant and reduces the growth of the detrimental organisms.

Farmers apply the chemicals as traditional practices to enhance the fertility of soil. However, it is well documented from last two decades that regular use of chemicals causes pessimistic impacts on soil bacteria, rhizobacteria and plant growth. Therefore, application of rhizobacteria to sustain nutritional status of a soil ecosystem is definitely an attractive and much desired ecological preference. To attain enviable special effects of plant growth rhizobacteria is a long-term strategy but a beneficial unconventional method to control. Due to its cost efficiency, eco gracious and ready

accessibility of renewable source of plant nutrients; it's found useful in maintaining long-term soil fertility and sustainability.

Sclerotium rolfsii is a most pathogenic soil-borne phytopathogen in the warm temperate regions all over the world. It causes many types of rot such as root rot, stem rot, foot rot, wilt rot in more than 500 species of plant including almost all crops (Farr *et al.*, 1989). The viability of this phytopathogen is due to production of special structure such as sclerotia that endure in soil for many years. Fungal virulence may be abridged by application of chemicals such as fungicides. On the other hand, continuous use of chemicals imparts direct effects on human health and to the biotic- abiotic components of ecosystem. Therefore, an ecofriendly approach is essential that could be much more environmentally acceptable and provide an alternative to the traditionally accessible chemical treatment methods.

The present study was premeditated to appraise the structural and functional diversity of rhizobacterial strains from rhizospheric zone of different plants and identify the various plant growth promotory metabolites that may enhances and maintains the system of agriculture in a much desired sustainable way.

Materials and Methods

Description of site and method of Sampling

The site of study was Sholapur district. It is defined as hot and semi-arid climatic zone. Five healthy plants were preferred on the basis of phenotypic characters from agricultural ground (45 days old). Bacteria from the rhizospheric zone of plants were collected. The choice of plants was as coriander, turmeric, potato, onion and

tomato. The plants and rhizospheric soil samples were collected in sterilized plastic bags and stored at 4⁰ C for further use.

Isolation of rhizospheric bacteria from rhizospheric zone of plants

Isolation of rhizobacteria was done according to Glick and Thomson (1993). Soil of non-rhizospheric zone was separated by vigorous shaking of the roots and physical parameters of soil (i.e. pH, temperature, moisture percentage) were checked according to the standard procedures. Plants roots were drenched in phosphate buffer saline in the flask and kept at incubator shaker at 250 rpm for 15 min at 37 ± 2⁰C. After incubation the suspension obtained was serially diluted and spread on nutrient agar plates and incubated at 37 ± 2⁰C for 24–48 hrs. After incubation, a well distinct single colony with distinct morphological characters was selected and maintained on nutrient agar slants for further studies. Generation time of the species calculated according to the standard methodologies. Rhizospheric diversity in the samples was calculated by Simpsons diversity index (H') according to Atlas and Bartha (1993).

Antagonistic property

Culture of *Sclerotium rolsfii*: The fungal pathogen was procured from Agararkar Research Institute (ARI), Pune. It was allowed to grow on Potato dextrose agar at 27 ± 2⁰ C and maintained at 4⁰C for further studies.

Antagonism *in vitro*: Dual culture technique was used to check the antagonistic activity of bacterial strains against phytopathogen as mentioned by Skidmore and Dickinson (1976). Mycelial discs of five-day-old with diameter 5 mm were

placed in four corners of the modified nutrient plates (supplemented of sucrose, 2%) and exponentially grown culture of rhizobacterial strains spotted at 2 cm just differing from the fungal disc and incubated at 37 ± 2⁰C for 120 hrs. Zone of inhibition was recorded by measuring the distance between the edge of fungal colonies and bacterial as compared to control (i.e. with rhizobacterial strains).

Assessment of plant growth promoting activities *in vitro*

IAA

The potentiality of rhizobacterial strains for production of indole acetic acid (IAA) was checked as per the methodology of Gordon and Weber (1951). Each of the rhizobacterial exponentially grown isolates were inoculated in respective media and incubated at 37 ± 2⁰C for 48 h at 150 rpm. After 72 hrs of incubation, in a ratio of 1:2 Salkowski's reagent was added to the culture broth and incubated at room temperature for 15–20 minutes.

A positive test was indicated by appearance of pink colour. Quantitative estimation of IAA production was carried out by measuring its absorbance at 530nm. Standard curve was prepared for IAA was prepared according to Koo and Cho (2009). The concentration of indole acetic acid produced by rhizobacterial strains was calculated from it.

Siderophore assay: Siderophore production by the rhizobacterial strains was estimated qualitatively on a universal medium Chrom-Azurol Siderophore (CAS) agar medium. Exponentially grown rhizobacterial strains were spotted on CAS agar medium plates and incubated at 37 ± 2⁰C for 48–72 h as described by Schwyn and Neilands (1987).

Hydrocyanic acid (HCN) production:

HCN production was checked according to Lorck (1948). Exponentially grown culture was streaked on nutrient agar plates supplemented with glycine (4.4 g l⁻¹). A filter paper was simultaneously soaked in picric acid, 0.5% (w/v) in 1% Na₂CO₃ and placed in the upper lid of the petri dish. After incubation at 37 ± 2° C change in color was observed. Positive test was indicated when color changes from yellow to orange. On the other hand, plates devoid of inoculum served as control.

Solubilization of phosphate: Ability of the rhizobacterial strains to solubilize phosphate (P) was checked in Pikovskaya agar medium. Log phase rhizobacterial strains were spotted in Pikovskaya agar plate and incubated at 37 ± C for 120 hrs. Formation of halo around the spotted colony indicates positive test. The solubilization index of the rhizobacterial strains was calculated by comparing the zone size including colony diameter. The quantitative estimation of phosphate was checked as per methodology of King (1932).

Solubilization of Chitin: Chitin solubilization was checked according to methodology of Mubarik *et al.* (2010). The log phase cultures of different rhizobacterial strains were spotted on chitin minimal medium agar plates. After incubation at 37 ± 2°C for 72 h, clear halo zone around the spotted strains indicates positive test.

Results and Discussion

Soil was found to be acidic in nature as pH was recorded in the range of 5.7±0.26⁰ C. The mean temperature during sampling month varied from 27.8±0.83⁰C. The moisture content of the soil was 17 ± 1 % during studies (Table 1).

From each rhizospheric soil, a single well distinct colony with different morphological characters was preferred for studies. On the basis of dissimilar morphological characters of the colonies, total 27 rhizobacterial isolates were isolated, 7 from Coriander, 5 from Onion, 6 from Potato, 4 from Tomato and 5 from Turmeric rhizospheric zones abbreviated as cor-1 to cor-7, oni-1 to oni-5, pot-1 to pot-6, tom-1 to tom-4 and tur-1 to tur-5. Exponential growth was observed after 24 hrs of incubation. All organisms were found to be fast growers with generation time more 3.2±0.4 h⁻¹.

To elucidate the rhizospheric community richness and evenness in the zones of different plants, diversity indices were calculated. It was observed that Simpson's diversity was found to be highest in Coriander plant followed by Potato and minimum in Tomato plant (Figure 1).

Plant growth promotory activities of rhizobacterial strains of different plants when assessed, showed that 100 % of the isolates were producers of IAA and solubilizers of phosphates (Figure 2). Maximum IAA producers were recorded from rhizospheric zone of Coriander; Onion and Potato plant i.e. 100 % followed by Tomato 50 % and Turmeric 20%. Maximum solubilizer of phosphate was recorded from rhizospheric zone of plant coriander, tomato, turmeric, onion and potato i.e. 100%. On the other hand, 70% of the isolates were producers of siderophore. Siderophore production was within range of 54 to 22 µg ml⁻¹. Maximum siderophores were obtained from rhizospheric soil of coriander i.e. 100 % followed by plant potato 83 %, onion 80%, tomato 50% and turmeric 74%. However, 74% were producers of HCN, bacterial strains from tomato and turmeric are 100 % producers of HCN, while 83% are

from potato, 71% are from coriander and 60% are from onion and turmeric. Only 18 % were chitin producers. High percent of chitin producers were reported from Tomato (50 %) followed by turmeric and potato 20% and coriander 16% (Table 2).

The isolates when screened for biocontrol potentiality it was observed that 66% of the isolates were able to control the growth of *Sclerotium rolsfii* efficiently. The rhizobacterial strains of Potato plant showed 83% of the potentiality in controlling growth of *S. rolsfii* followed by rhizobacterial strains of onion plant 80 %, tomato plant 75%, coriander plant 71 % and turmeric plant only 20 % efficient in inhibiting growth of fungal strain. Maximum growth controlling effect towards *S. rolsfii* was observed in the rhizobacterial isolates of Potato and Onion isolates. When combined PGPR traits were studied it was recorded that highest IAA, siderophore production and solubilization of phosphate was recorded in specifically cor-4 followed by tur-3 rhizobacterial strain (Figure 2).

Among all ecosystems the rhizosphere is defined as a most heterogeneous reservoir (Mittal and Johri, 2007). Heterogeneity arises due to the exudates released by plants in the rhizospheric zone. The 'rhizospheric effect' is a plant-dependent process that can be defined on the basis of rhizodeposition that is influenced by plant species (Hiltner, 1904). It is reported that the carbon assimilated by plants releases 10–40% of carbon in the soil as a form of root secretion. These secretions are composed of numerous organic compounds that act as chemo-attractants for bacteria of rhizosphere even at very low concentration (Philippe, 2006). Communities of rhizobacteria recovered from rhizospheric zones of different plants in present study depicted quite different phenotypic characters. It is also supported

by the values of H', which are greater for Coriander plant as compared to others. Similar findings are also reported earlier when diversity pattern from wheat, canola (Mittal and Johri, 2007) and Rice (Joshi *et al.*, 2011) are studied. The rhizospheric structural diversity depends upon an array of factors such as age of plant, breed of cultivars, characters of soil, its pH, content of organic matter, availability of nutrient, root metabolites (Campbell, 1985) and traditional practices like tillage, irrigation, fertilizer application, cropping, residue incorporation etc (Grayston *et al.*, 1996).

In the presented study, all the rhizobacterial strains of different plants produced a substantial amount of indole acetic acid. 100 % of organisms were producers of IAA. It is one of the most important phytohormones that are synthesized from amino acid tryptophan. It plays many important physiological functions for the plant including enlargement and division of the cell, root initiation, increased growth rate, differentiation of tissue, phototropism, geotropism and apical dominance (Khan *et al.*, 2009). It is well documented when IAA produced by the bacterial strains, stimulates development of the host plant root system (Sarwar and Frankenberger, 1994; Bharucha *et al.*, 2013). Diversity in the synthesis of IAA among rhizobacterial strains however is due to participation in various biosynthetic pathways, presence of enzymes to convert active free IAA into conjugated forms, change in conditions of environment, availability of precursor's and uptake of IAA by plants (Spaepen *et al.*, 2007). 70% of organisms were producers of siderophore. Siderophore is another important metabolite released by the PGPR strains that indirectly affects the growth of plants by two ways. Firstly microorganisms secrete siderophores to supply iron to plants for its uptake under iron-deficient conditions (Charest *et al.*,

2005). Secondly, it accordingly causes disease suppression by conferring an inadequate supply of essential trace minerals through which pathogen growth reduces drastically (Weller and Thomashow, 1993). It also directly stimulates the biosynthesis of

other antimicrobial compounds. Due to biosynthesis there is an increase in the availability of minerals to the bacteria that develops local and systemic host resistance in plants (Antoun *et al.*, 1998).

Table.1 Physical properties of the soil

Sr. no	Plant rhizospheric zones	Physical parameters		
		pH	Temperature (°C)	Total moisture content
1	Coriander	5.8	27	18
2	Turmeric	5.2	28	16
3	Potato	5.4	27	17
4	Onion	5.6	28	16
5	Tomato	5.8	29	18
	Mean	5.56	27.8	17
	Standard deviation	0.2	0.83	1

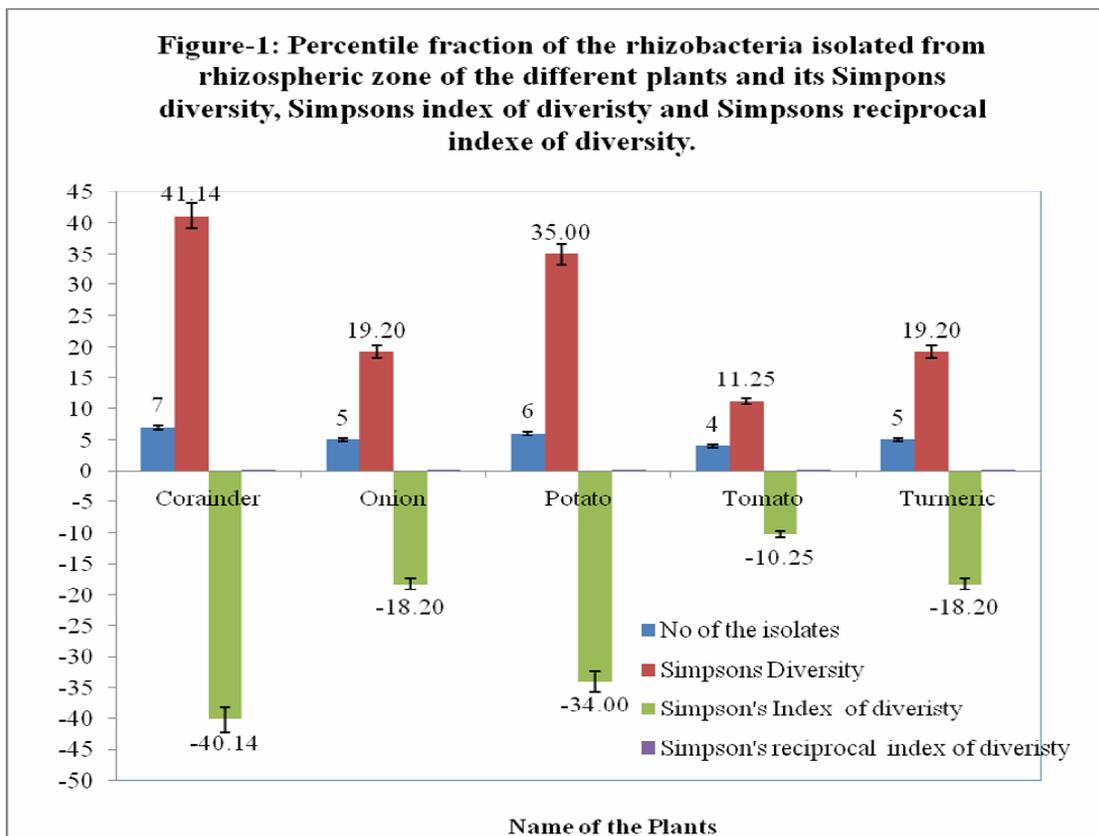
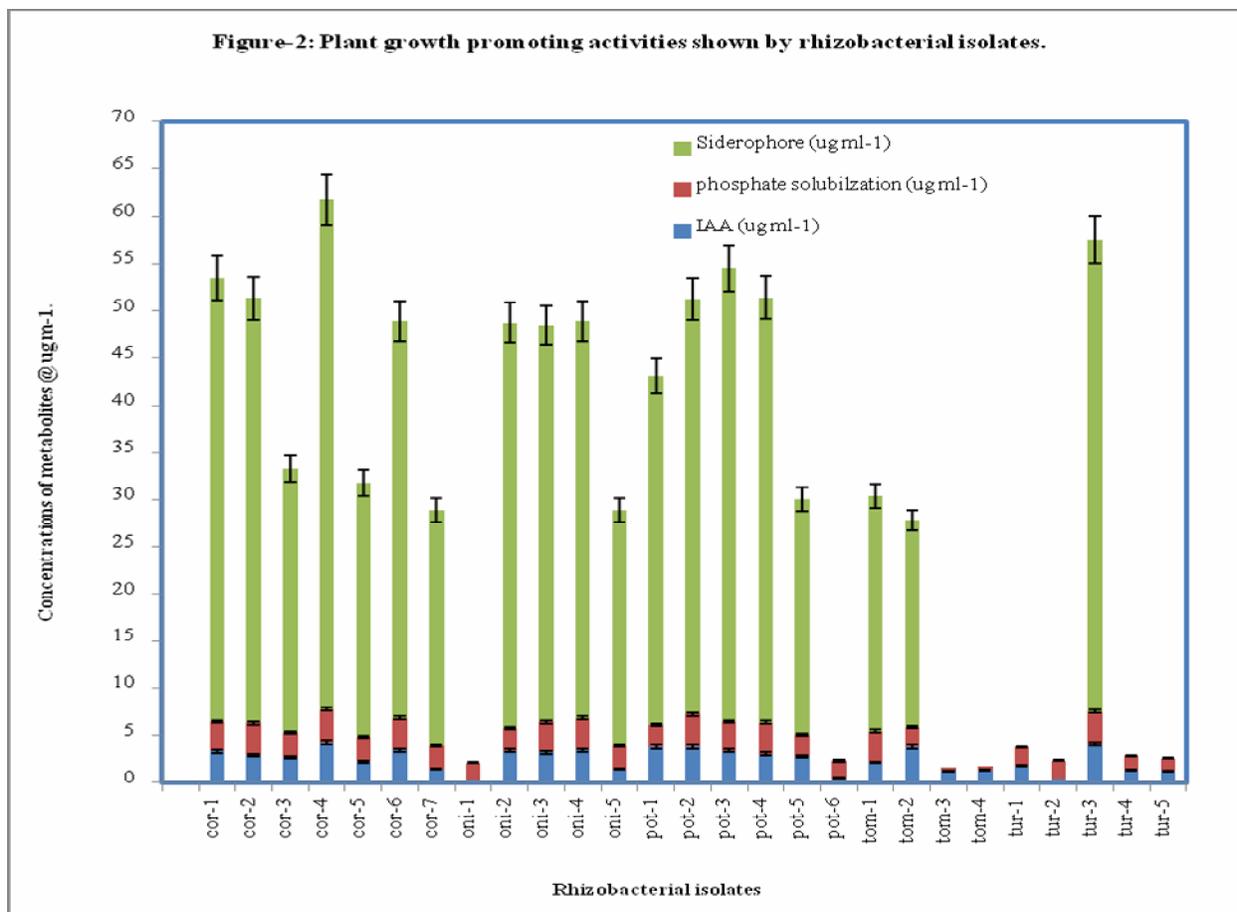


Table.2 Biocontrol and Plant growth and promoting activities of rhizobacterial strains

Sr no.	Rhizobacterial Strains	Production of HCN	Solubilization of chitinase	Production of siderophore	Biocontrol activity against <i>Sclerotium rolsfii</i> after incubation of	
					48 hr	120 hr
1	cor-1	+	-	+	+	+
2	cor-2	+	-	+	+	+
3	cor3	+	-	+	+	+
4	cor4	+	+	+	+	+
5	cor-5	+	-	+	-	+
6	cor-6	-	-	+	-	-
7	cor-7	-	-	+	-	-
8	oni-1	-	-	-	-	-
9	oni-2	+	-	+	+	+
10	oni-3	+	-	+	+	+
11	oni-4	-	-	+	+	+
12	oni-5	+	-	+	-	+
13	pot-1	+	-	+	-	+
14	pot-2	+	+	+	+	+
15	pot-3	+	-	+	+	+
16	pot-4	+	-	+	+	+
17	pot-5	+	-	+	-	+
18	pot-6	-	-	-	-	-
19	tom-1					
20	tom-2	+	+	+	+	+
21	tom-3	+	+	-	+	+
22	tom-4	+	-	-	-	-
23	tur-1	+	-	-	-	-
24	tur-2	+	-	-	-	-
25	tur-3	+	+	+	+	+
26	tur-4	-	-	-	-	-
27	tur-5	-	-	-	-	-

”+” indicates positive test (shows production of respective metabolite in the media, confirmed by the change in color formation or formation of halo zone around respective spotted colonies).

”-“ indicates negative test (shows no production of respective metabolite in the media, confirmed by the no change in color formation or absence of formation of halo zone around respective spotted colonies).



74% of the strains are HCN producers. The release of HCN by rhizospheric bacteria into the soil is documented to play a role in the suppression of soil pathogens by Gallagher and Manoil (2001). Phosphorus present in the soil is available in complex forms that are unavailable for uptake by plants (Khan *et al.*, 2009). In the present study 100 % of the rhizobacterial strains were found to be solubilizers of phosphate. The solubilization of insoluble P by the rhizosphere microorganisms is due to the concurrently decrease in pH of the medium due to secretion of organic acids (Freitas *et al.*, 1997). In the present study, 18% were chitin solubilizers. Chitinase is a group of enzymes that is only responsible for degrading chitin directly into low-molecular weight products. Chitin-degrading enzymes are of significant interest to chemical and pharmaceutical industries as may be applied as insecticide

and fungicide to control pests and fungal pathogens of plants respectively. It is well documented by workers that chitin solubilizing bacteria control the growth of phytopathogens as chitin is one of the most important constituent of their cell wall (Mezendorfer and Zimoch, 2003).

All these PGPR traits reported in present study were found to be positive in most of rhizobacterial strains. Earlier similar findings have also been documented by many workers that isolates from rhizospheric zone of different plants showed variations in PGPR activities (Kerry, 2000, Kumar *et al.*, 2013, Kumar *et al.*, 2014) which is found in with accordance of our study. On the other hand, present study also defines their importance as biological control agents.

In conclusion, results obtained on the basis of the study may be accomplished that rhizosphere has very important role in influencing structural and functional diversity. On the other hand, it also states that rhizobacteria acts as most potent striking and likely alternative for developing biocontrol agents against one of the most resistant phytopathogen *S. rolsfii*. The study also highlights the ability of rhizobacterial isolates to be used as successful inoculants for development of growth of plant. The major role of root exudates as the key compounds of the plant that plays important role in governing structural and functional diversity cannot be ignored and denied from the above presented research work.

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