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Functional Diversity of Soil Bacteria from Organic Agro Ecosystem

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ABSTRACT

Organic farming is an eco-friendly agro ecosystem that helps maintain soil health in terms of biological fertility and productivity and bacterial communities in soil play an essential role. A Model Organic Farm (MOF), developed in 2002 to demonstrate organic farming modules among the farming community, is utilized to study bacterial composition, diversity, seasonal variations, and plant growth promotion (PGP) traits of the soil. We observe rich bacterial diversity in MOF soil in terms of types and PGP traits. Rich bacterial diversity in MOF soil is represented by heterotrophs, coliforms, *Pseudomonas* spp., *Azotobacter* spp. and *Rhizobium* spp. and majority of these microorganisms display multiple PGP traits. Bacterial isolates are predominately positive to production of ammonia (NH₃) (93.2%), indole acetic acid (IAA) (89.6%), catalase (85.0%), 1-aminocyclopropane-1-carboxylate deaminase (ACCD) (78.6%) and siderophore (69.0%). Richness of their functional characteristics is further revealed by tolerance to salinity and wide range of pH. All the isolates are tolerant to > 5 % NaCl and wide range of pH. Furthermore, majority (96-97%) of the bacterial population of nitrogen fixers are *Azotobacter* spp. and *Rhizobium* spp. with multiple PGP traits, tolerance to salinity and wide range of pH. The present study demonstrates that organic farming enriches soil fertility and biodiversity, as well as, less dependent on external high inputs. Thus organic farming offers promise of achieving ecological, economic and social stability in food production system.

Keywords

Organic farming,
Bacterial diversity,
PGP traits, Salinity
tolerance, pH
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Introduction

Modern farming practices have negatively impacted not only agricultural productivity but also soil health, food quality and environmental pollution (Ram, 2003). Recently, alternative farming systems especially organic farming is becoming popular around the globe. Organic farming has various advantages over modern agricultural practices especially sustainability and environmental safety of agricultural

production. Sustainability and environmental safety of agricultural production relies on ecofriendly approaches like biofertilizers, biopesticides and crop residue return (Subramaniam *et al.*, 2014). Organic farming relies heavily on the natural breakdown of organic matter to replace nutrients taken from the soil by crops. These eco-friendly approaches foster soil microorganisms (Bohme *et al.*, 2005; Esperschütz *et al.*, 2007;

Singh *et al.*, 2015), favoring long term soil fertility (Berger *et al.*, 2013; Bhardwaj *et al.*, 2014), plant tolerance and crop productivity (Bhardwaj *et al.*, 2014), supports and strengthens productive and sustainable soil biological processes (Mader *et al.*, 2002) and emphasizes on soil-building programmes (Abraham, 2011). Applications of biofertilizers and manure compost in organic farming system influences structure and function of soil microbial community (Marschner *et al.*, 2003; Chu *et al.*, 2007; Gu *et al.*, 2009; Zhen *et al.*, 2014; Singh *et al.*, 2015) and the importance of microorganisms in the maintenance of quality and productivity of agricultural soils is unquestionable.

Sam Higginbottom University of Agriculture, Technology and Sciences (SHUATS) Model Organic Farm [SMOF] was developed during 2008 to 2017 under the National Project on Organic Farming (NPOF) to enthuse organic farming modules among the farming community. Under the research programme on organic certification standards of rice grown under SRI technology revealed satisfactory and promising findings with the acceptable productivity (Abraham, 2011; 2013) A corollary to high potential of organic farming for sustainable entrepreneurship by small land holders of Indo-Gangetic Plains of Asia-Pacific (Abraham *et al.*, 2013).

The regular monitoring of soil fertility of SMOF revealed several folds increase in macro- and micro-nutrients such as available organic carbon, nitrogen, phosphorous, potassium, sulfur, zinc, iron, magnesium and copper (Abraham, 2009, 2011, 2013; Debbarma and Abraham, 2015; Abraham *et al.*, 2016). However, information on bacterial composition, diversity, seasonal variations and plant growth promoting traits of SMOF is lacking and the present study is an effort in that direction. Our study reveals ecological, economic and social stability in food production through MOF.

Materials and Methods

SHUATS Model Organic Farm [SMOF]

SMOF, Allahabad is located at 25° 24' 42" N latitude, 81° 50' 56" E longitude and 98 m altitude above the mean sea level. It has a sub-tropical and semi-arid climate with the monsoon commencing from July and withdrawing by the end of September. About 1000 mm of mean annual rainfall is unevenly distributed and most of it is received during the sowing of kharif crops. Apart from this, a few winter and summer showers are also received.

Organic farming has been practiced for several decades on the campus of Sam Higginbottom University of Agriculture, Technology and Sciences, Allahabad. The SMOF covers an area of 2 hectares [5 acres] and it was further developed during 2008 to 2017 under the NPOF, which had a provision for certification, Lacon Quality Certification (P) Ltd. [Accreditation No. NPOP/NAB/006, Ministry of Commerce, Govt. of India] has been certifying SMOF during the past 7 years [Certificate No. ORG/SC/1009/001070] and is continued to-date.

Collection of soil sample

Soil samples were collected during the winter, summer and rainy seasons from the Organic Farm of SHUATS, Allahabad. The samples were placed in sterile plastic bags and kept at 40°C in the laboratory and analyzed within 4 h of collection.

Isolation of bacteria

Soil samples were serially diluted in sterile phosphate- buffered saline (Hi-media, pH 7.2) and inoculated on different media like Nutrient agar medium for total Heterotrophs, Kings media for *Pseudomonas* spp. (Ahmad *et al.*, 2008), Yeast Extract Mannitol Agar

(YEMA) for *Rhizobium* spp. (Vincent, 1970), Macconkey agar for coliform and Ashby's Agar for *Azotobacteria* spp. (Norris and Chapman, 1968). After incubation at 28-30°C for 24-48hrs, bacterial colonies were isolated. Bacterial cultures were maintained on slants at -20°C

Characterization of bacteria for plant growth promoting (PGP) traits

Bacterial isolates were characterized for PGP traits employing standard procedures. The following traits were analyzed.

Catalase production

Bacterial cultures were grown in nutrient agar medium for 18-24h at 37°C. The cultures were kept on clean slide with the help of loop and 2-3 drop mixed of H₂O₂ and observed the for gas bubbles. Organism producing gas bubbles was considered positive for catalase production (Schaad, 1992).

Ammonia production

Bacterial isolates were screened for the production of ammonia in peptone water. Freshly grown culture were inoculated in 10 ml peptone water in different tubes and incubated for 48-72 h. After 2 -3 d, Nessler's reagent (0.5 ml) was added in each tube. Development of brown to yellow colour was considered as positive for ammonia production (Cappuccino and Sherman, 1992).

Indole acetic acid (IAA) production

IAA production was detected following method described by Brick *et al.*, (1991). Bacterial cultures were grown in peptone water at 37°C for 72 h. Full-grown cultures were centrifuged at 3000 rpm for 30 min. The supernatant (2 ml) was mixed with 2 drops of orthophosphoric acid and 4 ml of Salkowski reagent (50 ml, 35% of perchloric acid, 1 ml

0.5 M FeCl₃ solution). Development of pink colour indicates IAA production.

HCN production

HCN was detected according to the method of (Lorck, 1948). King's medium was amended with 4.4 g glycine l⁻¹ and bacteria were streaked on agar plate. A Whatman filter paper no. 1 soaked in 2% sodium carbonate and 0.5% picric acid solution was placed at the top of the plate. Plates were sealed with parafilm and incubated at 37°C for 4 days. Development of yellow to red color on the filter paper indicated HCN production.

Phosphate solubilization (PS)

Phosphate solubilizations of isolates were evaluated from the ability to solubilize inorganic phosphate. Pikovskaya's agar medium containing calcium phosphate as the inorganic form of phosphate was used in the assay. A loop of bacterial culture was streaked on the plates and incubated at 28°C for 4-5 d. The appearance of transparent halo zone around the bacterial colony indicated the phosphate solubilizing activity of the bacteria (Nautiyal, 1999).

ACCD activity

ACCD activity was detected according to the method of Safronova *et al.*, (2006). The bacteria were grown in test tube containing 100 ml of liquid medium: KH₂PO₄(2g), K₂HPO₄(0.5g), MgSO₄(0.2g), Glucose (0.2g). The medium was supplemented with 0.3g ACC or 0.19g (NH₄)₂SO₄ as N-source and incubated for 24 -72h. The appearance of bacterial growth indicated the ACC deaminase activity of the bacteria.

Salinity tolerance

Isolated bacteria were inoculated in nutrient broth with different concentration of salt

(0.5%, 10%, 15% and 20%) and incubated for 48-72 h at 37°C. After 2-3 d growth of bacteria with respect to salt concentration was observed (Damodaran *et al.*, 2013).

pH tolerance

For determining pH tolerance of the isolated bacteria, they were inoculated in nutrient broth with varying pH (5, 6, 7, 8 and 9) and incubated 48-72 h at 37°C. Observations on bacterial growth were made after 3 d.

Statistical analysis

Statistical analysis was performed by Student's *t*-test for various comparisons.

Results and Discussion

SMOF soil displays bacterial abundance and diversity

Seasonal data reveals higher ($p < 0.01$) bacterial counts in soil from organic farm in summer as compared to winter and rainy season (Table 1). Total heterotroph count $8.4 \times 10^5 \text{g}^{-1}$ soil in summer declined to nearly half in winter and rainy season ($3.5\text{-}4.0 \times 10^5 \text{g}^{-1}$ soils). Though coliforms are not much studied in MOF soil, we have detected $1.7\text{-}6.0 \times 10^5$ CFU g^{-1} soil in the rainy season and 2 and 3.5 folds increase in winter and summer respectively (Table 1). The versatile *Pseudomonas* recorded higher count in the summer, close to Heterotroph counts. A significant number of *Pseudomonas* and *Azotobacter* spp. were also observed (Table 1).

The relationship between bacteria diversity and different season were determined by a biplot (Fig. 1). It is an enhanced scatterplot that uses both points and vectors to represent structure. The PCA biplot represents the variables with calibrated axes and observations as points allowing projecting the

observations onto the axes to make an approximation of the original values of the variables. The first two principal component axis of the biplot accounted for 71.1% (F1) and 19.4% (F2) of the total variation of the bacteria diversity and different season. In this biplot, bacteria diversity were located vary far from the origin of biplot, indicating strong bacteria diversity in different season. Eigen values of the first and second components were 2.135 and 0.583, respectively.

PGP traits among SMOF soil isolates

Bacterial isolates ($n=650$) from SMOF were screened for PGP traits and notably majority of them produced multiple PGP traits (Table 2). Isolates were predominately positive to production of NH_3 (93.2%), IAA (89.6), CT (85.0), ACCD (78.6%) and SD (69.0%). PS activity and production of HCN were detected in 38.0 and 27.6% isolates, respectively. Production of all PGP traits detected was higher among *Pseudomonas* spp. as compare to other bacterial groups isolated from organic farm (Table 2). Production of HCN by coliforms was lowest among all other groups of bacteria. Total heterotrophs were predominately positive to production of ammonia (97.3%), IAA (90.6%), siderophore (85.9%) and catalase (75.8%). About one third (27.5%) of them were ACCD and HCN producers and only in 19.4% PS activity was detected (Table 2).

Pseudomonas spp. was positive for catalase, production of ammonia and IAA more than 90% and ACCD and siderophore more than 80%. Production of HCN and PS activity was observed 61.3 to 54.5 % (Table 2). Of the 132 isolates of *Rhizobium* spp., 129 (97.7%) and 121 (91.6%) of them were positive to production of ammonia and IAA, respectively (Table 2). Production of Catalase and ACCD was detected in 117 (88.6%) and 112 (84.8%) of isolates of *Rhizobium* spp.

Production of ammonia and IAA was detected in 96.1% isolates of *Azotobacter* spp. followed by catalase, siderophore and ACCD (Table 2). PS activity (26.1%) and production of HCN (21.5%) were the least observed PGP trait in *Azotobacter* spp.

Similarly among coliforms production of ammonia, catalase, IAA and ACCD was detected in >72.8 % isolates (Table 2). PS activity and production of siderophore was detected in 40.1 and 32.7% of coliforms, respectively. However, production of HCN was detected in only 2.8% coliform isolates (Table 2).

Seasonal variations of PGP traits among all bacterial isolates from soil of SMOF are shown in Figure 3. In all the three seasons, majority (>60%) of heterotrophs were positive to production of ammonia, IAA, ACCD and catalase (Fig. 3A). However, in summer none of them showed siderophore activity and few of them (<5%) were positive to PS activity. In summer none of the heterotrophs was positive to production of HCN. As compared to rainy season significant number of heterotrophs were positive to siderophore ($p<0.001$) and PS activity ($p<0.01$) in winter season. All the coliforms were positive to catalase both in winter and rainy season and ammonia in summer season (Fig. 3B). Both in winter and summer around 80% of them showed production of IAA. ACCD activity in majority of coliforms was noted in summer season. Majority (>60%) of *Pseudomonas* spp. displayed all the PGP traits in all the three seasons except production of HCN and PS activity in winter (Fig. 3C). Production of ammonia and catalase was >95% isolates of *Rhizobium* spp. at all the three seasons (Fig. 3D). As compared to winter and summer, in rainy season production of ACCD, siderophore and PS activity was detected in significantly high number ($p<0.001$) of

isolates of *Rhizobium* spp. As the seasonal variations are concerned for the PGP traits among *Azotobacter* spp., production of HCN was noted lowest in all three seasons as compared to other PGP traits (Fig. 3E). Production of IAA in summer and PS activity in rainy season was in significantly high ($p<0.001$) number of *Azotobacter* spp. PS activity was noted least (54.5%) among all the PGP traits and also in all three seasons as compared to other PGP traits (Fig. 3E).

Majority (93%) of bacterial isolates from SMOF displayed multiple PGP (MPGP) traits (Table 3). Coliforms, *Rhizobium* spp. and *Azotobacter* spp. emerged as top displayer of MPGP traits followed by *Pseudomonas* spp. and heterotrophs (Table 3). Of the 650 total bacterial isolates examined, only 40 (6.1%) of them were without a single PGP trait. None of the bacterial isolate displayed single PGP trait and only 3 of them showed two PGP traits.

Different bacterial groups displaying MPGP traits are depicted in Figure 2. Overall large number of isolates representing different bacterial groups displayed five MPGP traits followed by four and six MPGP traits. Over 45% nitrogen fixer representing both *Rhizobium* spp. and *Azotobacter* spp. showed five MPGP traits. Similarly *Pseudomonas* spp. (44.6%) and coliforms (38.4%) displayed six and four MPGP traits, respectively.

Stress (salt and pH) tolerant PGPR

Bacterial isolates from soil of SMOF were studied for tolerance to salt and results are given in Table 4. Majority (74%) of the isolates were tolerant to 5% salt whereas 25% of them were tolerant to 10% salt. Only three bacterial isolates representing two coliforms and one isolate of *Azotobacter* spp. were tolerant to 20% salt. Except for *Azotobacter* spp. large number (>78%) of others organisms displayed tolerance to 5% salt

(Table 4). However, tolerance to 10% of salt was highest among *Azotobacter* spp. (48%) as compared to other bacterial isolates. Tolerance to pH among bacterial isolates from SMOF is given in Table 5.

The bacterial isolates from organic farm displayed tolerance to variable range of pH. Of the 650 bacterial isolates studied for their tolerance to pH range, 485 (74.6%) of them displayed tolerance to a wide range of pH 5-9 (Table 5). However, at neutral pH 7 very few 53(8.1%) of the isolates exhibited growth.

We observe a rich bacterial diversity in soil from MOF both in terms of their types and functional (PGP) traits (Table 1 and 2; Figure 1). In the past, researchers have reported increased soil bacterial biomass, activity and bacterial functional and taxonomic richness and diversity in organic farm (Gardner *et al.*, 2011; Lopes *et al.*, 2011; Das and Dkhar, 2011; Grantina *et al.*, 2011; Schmid *et al.*, 2011; Wang *et al.*, 2012; Stockdale *et al.*, 2013; Hartmann *et al.*, 2014; Lupatini *et al.*, 2017). These workers have also reported higher population of microorganisms in organic farm in comparison to conventional farms. Several meta-analyses have revealed positive effect of organic farming on biodiversity and increase in species richness (Rahmann, 2011; Tuck *et al.*, 2014).

Soil biodiversity is important for soil resistance and resilience (Girvan *et al.*, 2005; Brussaard *et al.*, 2007).

In the present investigation it observed rich seasonal bacterial diversity and biomass representing several bacterial groups such as heterotrophs, coliforms, *Pseudomonas*, *Azotobacter* and *Rhizobium* in MOF. We found a large number (69.0-93.2 %) of bacterial isolates with PGP traits and majority of them displayed multiple PGP traits except for HCN and PS activity.

Production of ammonia was observed predominantly among isolates from MOF. In an earlier study production of ammonia was detected in 74% bacterial isolates from rhizospheric soil of chickpea of conventional farm in the vicinity of Allahabad (Joseph *et al.*, 2007) whereas the present study showed higher ammonia producers (93%) from the organic farm of the same vicinity. In addition to the source of nitrogen in soil its involvement in antagonistic interactions with soil pathogens that result in disease control is reported (Saraf *et al.*, 2008). Narula and Gupta (1986) found that inoculation of wheat and barley with ammonia excreting strains caused increased dry weight and enzyme activity.

Catalase is a very important enzyme in protecting the cell from oxidative damage by reactive oxygen species (ROS). It is well known that the products of oxygen reduction such as hydrogen peroxide can be highly toxic for cells but catalase can split hydrogen peroxide into molecular oxygen and water, preventing cells from damage by reactive oxygen species (Yao *et al.*, 2006).

Table.1 Bacterial Diversity of soil from SMOF

Organisms	Total Viable Count (CFU x10 ⁵ g ⁻¹)		
	Winter	Summer	Rainy Season
Heterotrophs	4.0	8.4**	3.5
Coliforms	3.4*	6.0**	1.7
<i>Pseudomonas</i> spp.	3.6**	8.2***	1.4
<i>Azotobacter</i> spp.	3.3	7.2**	2.5
<i>Rhizobium</i> spp.	4.0	7.6*	2.8

CFU=Colony forming unit, *p<0.05; **P<0.01; ***p<0.001

Table.2 Plant growth promoting (PGP) traits in soil bacteria from SMOF

Organism	No.	Incidence of PGP Traits (%)						
		NH ₃	HCN	SD	IAA	ACCD	PS	CT
Heterotrophs	149	145(97.3)***	41(27.5)	128(85.9)***	135(90.6)***	106(27.5)**	29(19.4)	113(75.8)**
Coliforms	107	86(80.3)***	3(2.8)	35(32.7)**	80(74.7)***	78(72.8)***	43(40.1)**	86(80.3)***
<i>Pseudomonas</i> spp.	132	121(91.6)***	81(61.3)**	111(84.0)**	122(92.4)***	118(89.3)**	72(54.5)*	123(93.1)***
<i>Rhizobium</i> spp.	132	129(97.7)***	27(20.45)	76(57.6)**	121(91.6)***	112(84.8)***	65(49.2)**	117(88.6)***
<i>Azotobacter</i> spp.	130	125(96.1)***	28(21.5)	99(76.1)**	125(96.1)***	97(74.6)**	34(26.1)	112(86.1)***
Total (%)	650	606(93.2)***	180(27.6)	449(69.0)**	583(89.6)***	511(78.6)***	243(38.0)*	551(85.0)***

*p<0.05; **P<0.01; ***p<0.001, Production of ammonia (NH₃), hydrogen cyanide (HCN), Siderophore (SD), indole acetic acid (IAA), 1-aminocyclopropane- 1-carboxylate deaminase (ACCD), phosphorus solubilization (PS) activity and Catalase (CT)

Table.3 Number of PGP Traits among bacterial isolates from SMOF

Organisms	No. of isolates	No. of PGP traits (%)			
		0	1	2	>2
Heterotrophs	149	16(10.7)	00	01(0.6)	132 (88.5)
Coliform	107	02(1.8)	00	02(1.8)	103 (96.2)
<i>Pseudomonas</i> spp.	132	10(9.3)	00	00	122 (92.4)
<i>Azotobacter</i> spp.	130	06(4.6)	00	00	124 (95.3)
<i>Rhizobium</i> spp.	132	06(4.5)	00	00	126 (95.4)
Total	650	40(6.1)	00	03(0.4)	607(93.3)

Table.4 Salt tolerance among bacterial isolates from SMOF

Organisms	No. of isolates	No. of organisms tolerant to Salt (%)		
		5	10	20
Heterotrophs	149	125(83.8)	24(16.1)	0
Coliform	107	84(78.5)	23(21.4)	2(1.8)
<i>Pseudomonas</i> spp.	132	105(79.5)	26(19.6)	0
<i>Rhizobium</i> spp.	132	103(78.0)	29(21.9)	0
<i>Azotobacter</i> spp.	130	66(50.7)	63(48.4)	1(0.7)
Total	650	483(74.3)	165(25.3)	3(0.4)

Table.5 Tolerance to pH among bacterial isolates from SMOF

Organisms	No. of isolates	No. of organisms tolerant to pH (%)						
		5-9	5-8	6-9	6-7	7	7-9	7-8
Heterotrophs	149	123(82.0)		24(16.1)	0	0	0	2(1.3)
Coliform	107	83(77.5)	1(0.9)	5(4.6)	2(1.8)	5(4.6)	3(2.8)	8(7.4)
<i>Pseudomonas</i> spp.	132	107(81.0)	0	6(4.5)	0	9(6.8)	4(3.0)	6(4.5)
<i>Rhizobium</i> spp.	132	88(66.6)	0	1(0.7)	3(2.2)	33(25.0)	5(3.7)	2(1.5)
<i>Azotobacter</i> spp.	130	84(64.6)	0	23(17.6)	0	7(5.3)	3(2.3)	13(10.0)
Total	650	485(74.6)	1(0.1)	57(8.7)	5(0.7)	53(8.1)	14(2.1)	28(4.3)

Fig.1 PCA biplot of different groups of bacteria at three different seasons under organic farming

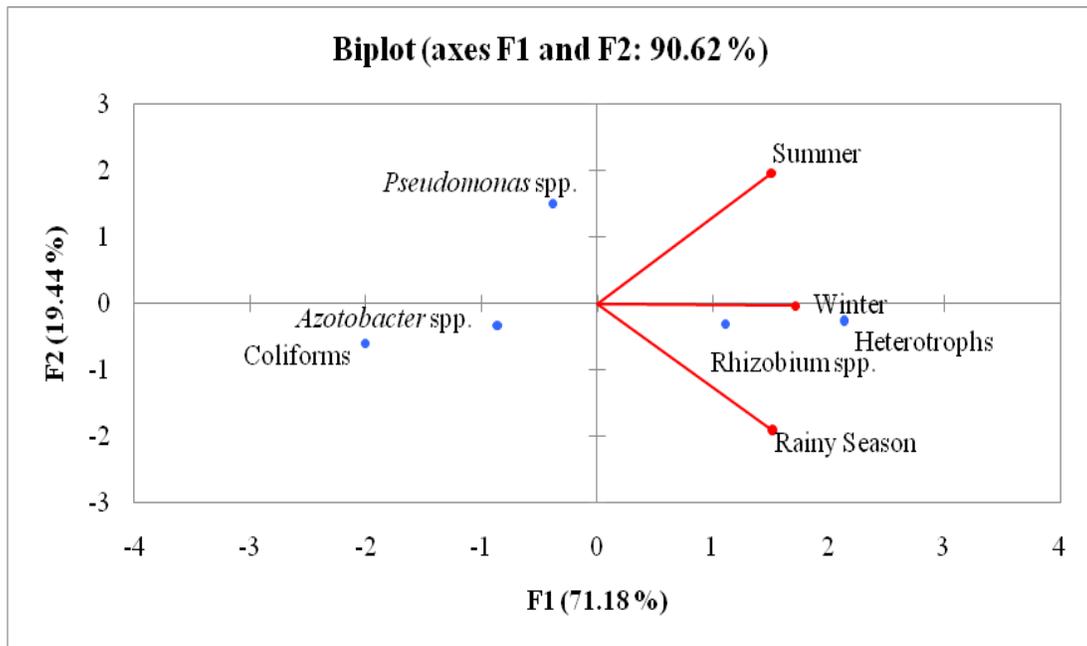


Fig.2 Distribution of MPGP traits among different bacterial groups

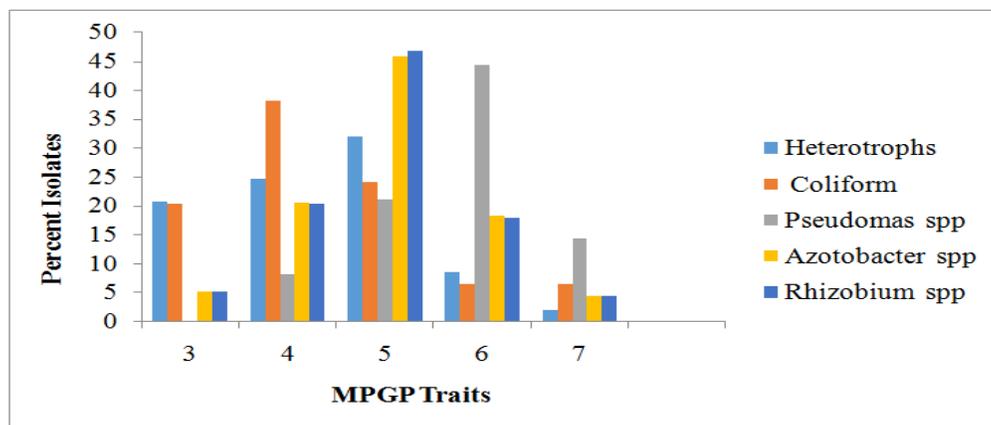
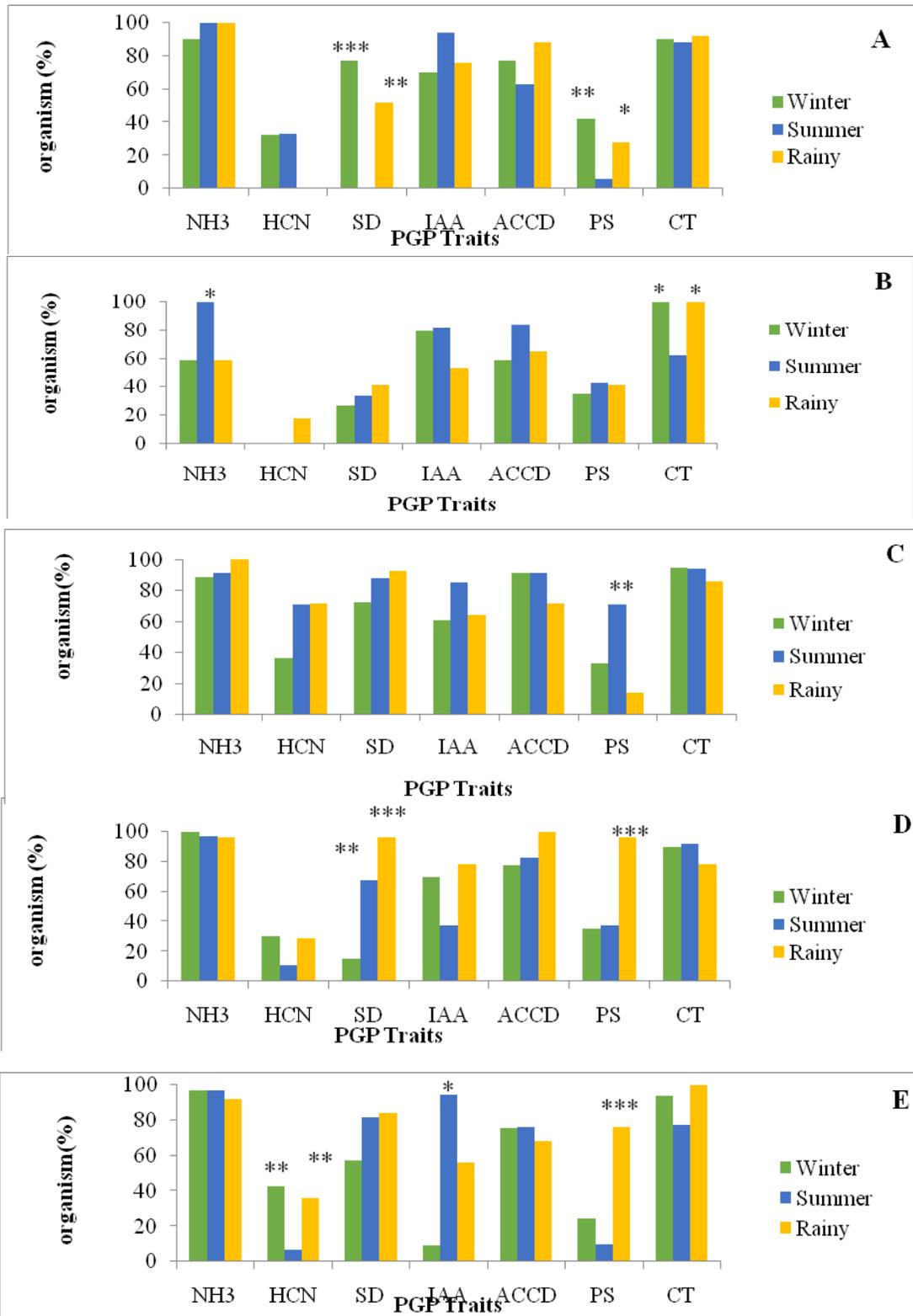


Fig.3 Seasonal variation of PGP traits in bacterial isolates from



*p<0.05; **P<0.01; ***p<0.0001, A) Heterotrophs, B) Coliform, C) *Pseudomonas* spp., D) *Rhizobium* spp., E) *Azotobacter* spp. Production of ammonia (NH₃), hydrogen cyanide (HCN), siderophore (SD), indole acetic acid (IAA), 1-aminocyclopropane- 1-carboxylate deaminase (ACCD) and phosphorus solubilization (PS) activity

Production of IAA is widespread among rhizobacteria (Patten and Glick, 1996; Khalid *et al.*, 2004; Spaepen *et al.*, 2007) and is one of the most physiologically active auxin or phytohormones implicated in the regulation of plant growth and development. Researchers have observed an increase in root hairs and lateral roots thus increasing the total root surface leading to an enhanced mineral uptake from the soil by inoculation of plants with PGPR with ability to produce high levels of IAA (Patten and Glick, 2002; Aloni *et al.*, 2006; Fukaki *et al.*, 2007). PGPR mediated induction of seed germination and root and shoot length was demonstrated by several researchers (Anitha and Das, 2011; Ramteke *et al.*, 2012).

In addition to its effect on plant growth, microbially produced IAA has been suggested to trigger an increased level of protection against external adverse conditions by coordinately enhancing different cellular defense systems. Because of this multiple effects on plants, many pathways such as tryptophol, tryptamine, indole-3-pyruvic acid and indole-3-acetamide pathways have been reported to be evolved in microorganism for IAA production (Gravel *et al.*, 2007). Thus, IAA production-based screening can be considered as an effective tool for detecting beneficial microorganisms with regulatory effect on plant growth (Ali and Hasnain, 2007; Govindarajan *et al.*, 2007). Microbes play an important role in the acquisition and transfer of nutrients in soil (Richardson, 2001). Therefore, the utilization of soil microbes to activate minerals and enhance nutrient uptake in plants has attracted increasing attention in sustainable agriculture (Fayez and Mahmoud, 2006). Phosphorus (P) is one of the major essential macronutrients for plants. In India, it is estimated that there are almost 260 million tons of phosphate rock deposits and this material should provide a cheap source of phosphate fertilizer for crop

production (FAI 2002). Although in soil P is available abundantly, its bioavailability in soil remains low due to the chemical transformations of P into insoluble forms (Rodriguez and Fraga, 1999) and thus a major constraint to the plant growth and crop production (Chiquito-Contreras *et al.*, 2012). Phosphate-solubilizing bacteria (PSB) have been considered as one of the possible alternatives for mediating inorganic phosphate solubilization and increasing its availability to the plants (Rodríguez *et al.*, 2006).

In the present study, a significant number of organisms (38%) displayed phosphate solubilizing activity. A study by Kaur and Reddy (2014) suggested that PSBs play an important role in improving crop productivity in organic farming. They noted significant increase in the biometric parameters (shoot height, shoot and root dry biomass) of maize and wheat plants after treatment with PSBs. Similar results were noted by other investigators (Dugar *et al.*, 2013; Hassimi *et al.*, 2013; Ranjan *et al.*, 2013). In addition to PS activity, PSBs may also improve the plant productivity by producing other secondary metabolites. There are several evidences related to plant growth promotion by PSBs through the production of indole acetic acid (IAA) and siderophores (Hariprasad and Niranjana 2009). Few earlier studies established the relationship between P-Solubilizing Index (PSI) and growth and physiological parameters of Phosphate solubilizing endophytes (Parihar *et al.*, 2003; Parihar and Ramteke, 2003).

Iron (Fe) is an essential plant micronutrient and microbial siderophores enhance Fe uptake by plants (Kloepper *et al.*, 1980; Katiyar and Goel, 2004; Dimkpa *et al.*, 2009) and thus plays an important role in plant growth promotion. Although large portion of Fe is present in soil, it acts as a limiting factor for

plant growth because its existence in the form of highly insoluble ferric hydroxide. Bacteria secrete siderophores to solubilize Fe from their surrounding environments by forming a complex ferric-siderophore and provide it to the plants for growth promotion (Andrews *et al.*, 2003). Siderophore mediated suppression of phytopathogens was reported by Jasim *et al.*, (2015). Here, a physiological response is implied by the fact that HCN production is induced by iron (Bakker and Schippers, 1987; Keel *et al.*, 1989; Voisard *et al.*, 1989) and that it is under the strong influence of quorum sensing (Pessi and Haas, 2000). The latter is likely to happen particularly in the rhizosphere, where root exudation promotes high bacterial counts.

The enzyme 1-aminocyclopropane-1-carboxylate deaminase (ACCD) is widely spread in bacteria (Belimov *et al.*, 2001, Ghosh *et al.*, 2003; Ma *et al.*, 2003; Dey *et al.*, 2004; Glick, 2005; Hontzeas *et al.*, 2005; Blaha *et al.*, 2006; Madhaiyan *et al.*, 2006; Duan *et al.*, 2009). It cleaves ACC, the immediate precursor of ethylene in plants and convert into ammonia and α -ketobutyrate (Glick *et al.*, 1998). These products of ACC cleavage are potential nitrogen and carbon sources (Glick *et al.*, 1998) that can play a role in the microorganism's fitness under stressful situations. Under stress conditions plants produce higher levels of the phytohormones ethylene, which means that the plants also produce higher levels of ACC (Glick *et al.*, 2007).

Under stress conditions, not only plant produce increased amount of ACC, the vast majority of rhizosphere microorganisms produce the phytohormone indole acetic acid (IAA) which acts to loosen plant cell walls thereby facilitating root exudation. In addition, bacterial IAA production has been shown to increase ACC synthase expression in plants (Kende, 1993). Thus,

microorganisms that can produce IAA and utilize ACC may have a competitive advantage over other soil microorganisms (Glick *et al.*, 1998, Stearns *et al.*, 2012). It is known that ACCD modulates ACC metabolism and is related to plant growth promotion and microorganism's developmental processes (Nascimento *et al.*, 2014) and thus protecting the plant from stress (Subramanian *et al.*, 2015).

Studies reveal the role of ACCD in influencing the senescence of flowers and thereby increasing the shelf life (Ali *et al.*, 2012; Jasim *et al.*, 2015). Additionally, recent results suggest a considerable degree of horizontal ACCD gene transfer events (Nascimento *et al.*, 2014). Hence, exploration of more ACCD gene from diverse bacterial sources and in depth understanding of the function of this enzyme especially in plant physiology signifies its importance. This may be the key molecule in a variety of important agricultural and biotechnological applications of ACCD genes and their expression (Nascimento *et al.*, 2014).

Volatile compound HCN produced by rhizobacteria is mainly considered as a biocontrol agent against phytopathogenic fungi (Ramette *et al.*, 2003; Ahmad *et al.*, 2008; Rezzonico *et al.*, 2007; Siddiqui *et al.*, 2006).

It is proposed that HCN interrupts functioning of many enzymes (Cooper and Brown, 2008) or protein carriers and as a result inhibits the growth of certain organisms. Wei and co-workers (1991) opined the possibility of HCN inducing systemic resistance in some plants making them resistant to phytopathogen attack (Siegie'n and Bogatek, 2006).

Contrary to the role of HCN as abiocontrol agent, several researchers concluded that HCN is hardly a universal biocontrol agent

and even caused phytotoxic effects in most in vitro experiments (Alström and Burns, 1989; Pal *et al.*, 2000; Kremer and Souissi, 2001; Rudrappa *et al.*, 2008; Blom *et al.*, 2011). In a recent study by Rijavec and Lapanje (2016) have shown that there is no correlation between the amount of HCN produced by a particular strain and its ability to inhibit the growth of phytopathogenic bacteria or fungi. These workers proposed a new role for HCN production by rhizospheric bacteria. HCN seems to increase phosphate availability for rhizobacteria and plant hosts especially in oligotrophic alpine environments. Richness of their functional characteristics is further revealed by their tolerance salinity and wide range of pH. Soil salinity and extreme pH are matter of serious concern for agricultural productivity. Use of salt tolerant PGPR is an effective approach to enhance growth and tolerance of various crops under salt stress conditions (Sharma *et al.*, 2016). In the present study all isolates from organic farm were tolerant to > 5 % NaCl and wide range of pH. Biologically nitrogen fixing organisms have a very important role in any agro-ecological system due to their ability to convert atmospheric nitrogen into fertilizer. In the present study we obtained major (96-97%) constituent of bacterial population of nitrogen fixers *Azotobacter* spp. and *Rhizobium* spp with multiple PGP traits and tolerance to salinity and wide range of pH.

Organic farming adopts a sustainable production practices in which low input system is aimed at mitigation of negative impact of conventional high input farming practices such as use of synthetic fertilizers, pesticides and other inputs (Gomiero *et al.*, 2011).

It combines scientific knowledge of ecology and modern technology with traditional farming practices based on naturally occurring biological processes. Since organic

farming avoids the inputs of synthetic chemicals, the build-up of a large and active soil microbial biomass is, therefore, critically important for sustaining the productivity of soils in organic farming systems (Tu *et al.*, 2006).

Although crop productivity of organic farming is found reduced in the initial stages as compare to conventional farming practices that uses heavy chemical fertilizers and pesticides, in due course of time organic farming practices do give better results and helps positively in sustenance of soil health and microflora, which indirectly maintains the balance of nature. Organic farming system substantially reduces the use of synthetic fertilizers, pesticides, energy and mechanic stress and aims at mitigating negative impacts of high-input farming practices in order to improve sustainable production (Gomiero *et al.*, 2011). Over the years, organic systems revealed an increase in microbial biomass and activity, largely driven by quantity and quality of farmyard manure (Fliessbach *et al.*, 2007; Birkhofer *et al.*, 2008).

The studies demonstrated the clear difference between soil microbiom associated with organic amendments and chemical fertilizers (Ling *et al.*, 2016). Organic fertilizer applications interact with soil chemical properties and boost soil fertility thereby significantly impacting the associated soil biodiversity making ecosystem more resilient to stress (Liu *et al.*, 2016; Ling *et al.*, 2017). Most functional groups involving nutrient related metabolism and recycling at significantly high abundance in organic amended soil. Changes in soil environmental conditions exert strong effects on microbial diversity, phylotype composition and ecosystem function (Xun *et al.*, 2016).

Long term organic amendments support stronger functional potentials of organic farm

system. Other organic amendment benefits are soil stability and buffering capacity (Ling *et al.*, 2016), increase in soil carbon stocks (Xie *et al.*, 2014) and improvement in soil structure and water retention (Yu *et al.*, 2012), soil enzyme activities (Bowles *et al.*, 2014; Kotroczo *et al.*, 2014), and that they simultaneously provides maintenance of soil health (Chaparro *et al.*, 2012) and suppression of soil-borne diseases (Qiu *et al.*, 2012). Diverse types of long-term fertilization management at the study site exert significantly different effects on soil physical and chemical properties (Xun *et al.*, 2016). In the long run organic farming system emerges as a sustainable adaptive agricultural practice that is less dependent on external high inputs and maintains the productive agro ecosystem that will meet our food and nutritional requirements.

The present study demonstrated that organic farming system enriches soil bacterial diversity and soil fertility and rendering the agro ecosystem less dependent on external high inputs. We observe rich bacterial diversity in MOF soil both in terms of their types and functional (PGP) traits. Bacterial isolates were predominantly positive to production of ammonia, IAA, catalase, ACCD and siderophore. Richness of their functional characteristics further revealed by their tolerance to salinity and wide range of pH. Organic farming system offers huge promise of achieving ecological, economic and social stability in food production system. However, organic farming is faced with a need to expand and develop in line with increasing demands for food and growing environmental concerns.

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