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SCREENING AND CHARACTERIZATION OF BACTERIAL FLORA FOR PGPR ACTIVITIES ISOLATED FROM UPLAND RICE VARIETIES

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Abstract

Increased use of pesticides and fertilizers are the major cause of global warming and ground water pollution. Use of microbial inoculants is an environmentally safer alternative in order to meet the increasing demand of fertilizers and thus for sustainable food production. In the present work a total of fourteen endophytic isolates were purified from root tissue of four upland rice varieties. These were further subjected for the screening of plant growth promotion traits *i.e*. IAA production, Siderophore production, Phosphate solubilization, Zinc solubilization, HCN production, nitrogenase activity. *In vitro* Screening of the isolates to check for antagonism against *Xanthomonas oryzae* and *Rhizoctonia solani* was also carried out.

Among the 14 isolates tested, 3 isolates (MKA3, MKA12, MKA7) were positive for siderophore production, 2 isolates (MKA10, MKA11) were positive for phosphate solubilization, 8 were positive for production of IAA. The maximum amount of IAA was produced by MKA3 (25±0.83µg/ml). Seven isolates tested positive for ARA activity with nitrogenase activity in the range of 20-120 n moles ethylene produced /h/mg protein. Two of the isolates (MKA1 & MKA3) were antagonistic against *Xanthomonas oryzae* and one (MKA3) against fungus *Rhizoctonia solani*. 16S rDNA sequencing of the 14 isolates showed 99-100% homology to different *Bacillus* sp.. These isolates harboring various PGP traits can be used as potential biofertilizers.

Key words: Fertilizers, Global warming, microbial inoculants, endophytic, upland.

Introduction

Rice is the staple food for more than a half of the world population (FAO, 2013). With the world's fast growing population, ensuring a sustainable food production is a major challenge. The demand of rice will continue to grow at a pace that exceeds growth in supply. Rice is probably the most diverse crop which is grown in different environmental conditions and categorized as upland, lowland, irrigated and rainfed. The categorization is based on several criteria, including water regime, drainage, soils and topography. Rice production is affected by several factors which includes climate, physical conditions of soil, water management, soil microbiome, soil fertility, cultivar, weed control and fertilization. Increased use of pesticides and fertilizers are the major cause of global warming and ground water pollution (Naher *et al.*, 2015). To meet the world's demand for rice it is imperative to find environmentally sound ways that supplement the need for fertilizers (Yasmin *et al.*, 2009). Use of microbial inoculants is an environmentally safer alternative in order to meet the increasing demand of fertilizers and thus for sustainable food production.

Soil microbiome is an important factor affecting the overall growth and productivity of the rice plant (Luo *et al.*, 2016). Close association of microorganisms with plants exert various kinds of positive effects on plant health. Rhizosphere bacteria, which live in the soil that is in intimate contact with the roots, are able to perform beneficial functions and these are known as plant-growth promoting rhizobacteria (PGPR) (Bhattacharyya and Jha, 2012). Some rhizospheric bacteria are capable to penetrate deep inside the surface of the roots and colonize the internal tissues and these are called endophytes (Moronta-Barrios, 2018). These endophytes reside inside the host plant tissue and provide various benefits to it particularly plant growth promotion and protection from pathogens under diverse environmental conditions (Santoyo *et al.*, 2016).

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PGPR promote plant growth by several mechanisms which include induced systematic resistance, production of plant growth regulators like indole acetic acid, gibberellic acid, cytokinins and ethylene, asymbiotic nitrogen fixation, antagonism against phytopathogens *via* siderophores, antibiotics and cyanide, fungal cell wall lysing enzymes which suppress the growth of fungal pathogens, solubilization of mineral phosphates and other nutrients. Also, the role of plant-associated microbiomes in plant adaptation to drought stress is emerging (East, 2013). ACC deaminase producing bacteria is one such kind which have the ability to mitigate the drought stress by lowering the ethylene levels.

Keeping these facts in view, the present study deals with screening and characterization of bacterial flora isolated from four upland rice varieties for plant growth promotion traits.

Materials and Methods

Isolation of endophytes

Four upland rice varieties *viz*. Nagina-22, APO, NERICA-L and MAS-946-1 were procured from the field of ICAR-IARI, PUSA, New Delhi for isolation of endophytic population from rice roots. For isolation, the roots were collected at crop flowering stage, surface sterilized using mercuric chloride solution (0.1%) and crushed in sterile water. The extract was plated on NA, King's B and Jensen media. The colony growth was observed after 72h of incubation and single colonies were maintained as pure isolates accordingly.

Identification of Endophytic isolates

For the identification of bacterial endophytes, 16SrDNA was sequenced for which genomic DNA was extracted using Zymo bacterial gDNA isolation kit and amplified using 16S universal primer pair forward (5'AGAGTTTGATCCTGGCTCAG3') and reverse (5'AAGGAGGTGATCCAGCCGCA3') primers under standard conditions. PCR mixture contained 50 ng of template DNA, primers of 10 pmol concentration with PCR master mix containing Taq DNA polymerase, dNTPs and MgCl2. The reaction conditions were : 1 min at 94°C, 1 min at 55°C and 1 min 50 sec at 72°C for 35 cycles. The expected size of amplicon was 1.5 kb. PCR products were purified using nucleopore PCR purification kit and further subjected to sequencing and submission of sequences to NCBI.

Plant growth promoting activities of endophytes

IAA production: For the determination of IAA production, the isolates were inoculated in nutrient broth (NB) supplemented with and without tryptophan (10µg/ml) and incubated at 28±2°C for 2days. After incubation period, cultures were centrifuged at 5000 rpm for 10 minute. Two ml of supernatant was mixed with two drops of orthophosphoric acid and 2 ml Salkowski's reagent (50 ml, 35% perchloric acid; 1 ml 0.5M FeCl3). Pink to red colour development indicates the production of IAA by the isolates. The optical density (OD) was recorded at 530 nm using a spectrophotometer. The amount of IAA was estimated by standard IAA graph and expressed as µg/mL (Patten and Glick, 1996).

Phosphorus solubilization

Phosphate solubilization by endophytes was evaluated by doing spot inoculation on tricalcium phosphate containing Pikovskaya (Pikovskaya, 1948) agar media plates. The plates were incubated at 28 ± 2°C for 5-7 days. Appearance of a clear zone around endophyte colonies indicated the P-solubilization ability by the isolates.

HCN production

To check the HCN production ability of endophytes, Castric's method (Castric, 1975) was followed. All endophytic bacteria were grown in 10% tryptone soy agar supplemented with glycine (4.4 g/1). Whatmanfilter paper No. 1 soaked in 2% sodium carbonate and 0.5% picric acid solution was placed to the underside of the Petri dish lids and the plates were sealed with parafilm and incubated at 30°C for 5-7 days. Change in colour of filter paper from yellow to red-brown indicates HCN production.

Siderophore production

Nutrient agar medium supplemented with chrome azurol S (CAS) dye solution (Milagres *et al.*, 1999) was used to check the siderophore production by the isolates. Spot inoculation was done for each isolate on plates and these plates were incubated at 30°C for 5 days. The presence of orange halo zone around the colonies was taken as a positive test.

In vitro Screening of bacteria for Antagonistic activity

Against *Xanthomonas oryzae* pv. *oryzae:* All the 14 isolates were screened for their antagonistic activity by dual culture assay against the bacterial sheath blight phytopathogens *Xanthomonas oryzae* pv. *oryzae.* The pathogen was first grown in LB overnight and subsequently spread on LA plates followed by spot inoculation of all the 14 isolates. Plates were incubated at 30°C for 5 days to visualize the formation of zone of inhibition.

Against *Rhizoctonia solani*

The isolates were screened for their biocontrol

activity against fungal sheath blight phytopathogens *Rhizoctonia solani* AG1 (Division of Plant Pathology, IARI) using dual culture assay (Sakthivel and Gnanamanickam, 1986). Isolates were grown on PDA media along with the fungus to screen for their antagonistic activity. Plates were incubated at 30°C for 7 days to observe the inhibition of the growth of fungus.

Screening for diazotrophic community

Screening on Nitrogen free media: Isolates were screened for their ability to grow in nitrogen deficient media composed of Glucose 10g/l, K_2HPO_4 0.1g/l, KH_2PO_4 0.4g/l, $MgSO_4$ 0.2g/l, NaCl 0.1g/l, $CaCl_2$ 0.02g/l, $FeCl_3$ 0.01g/l, $NaMoO_4$ 0.002g/l, Agar 18g/l for solid medium. The pH of the medium was adjusted to 7 ± 0.1. Isolates were first grown in Nutrient broth overnight at 30°C at 220rpm. The Culture was harvested, washed with N free broth two times and inoculated in N free media plates. Plates were incubated for 48hrs. A total of 3 sub culturing was done to check for the growth of isolates on Nitrogen free media plates.

***nifH* gene Amplification**

Amplification of *nifH* gene was carried out using gDNA as template using primer pair forward (5'GCIWTHTAYGGIAARGGIGGIATHGGIAA 3') and reverse (5'ATIGCRAAICCICCRCAIACIACRTC3'). PCR mixture contained 50 ng of template DNA, primers of 10 pmol concentration, Taq DNA polymerase, dNTPs and $MgCl_2$. The PCR conditions were: 1 min at 94°C, 1 min at 58°C and 1 min 50 sec at 72°C for 35 cycles. The expected size of amplicon was 410bp. Amplified products were purified using nucleopore PCR purification kit and further sequenced. Sequences were submitted to NCBI.

Nitrogenase activity

Acetylene reduction assay (ARA) was done for the examination of nitrogenase activity of endophytes. Each endophyte culture was inoculated in 25 ml capped tube containing 5 ml of nitrogen free semisolid agar media. The 10% volume of capped tube was replaced with 10% acetylene (v/v) and incubated at 30°C for 24 hours. Ethylene formation was measured by gas chromatography. The nitrogenase activity was calculated in unit nmol C_2H_4/ mg protein/h (Lee and Yoshida, 1997).

Results and Discussion

Isolation and Characterization of endophytic isolates

Four different upland varieties of rice *i.e.* Nagina-22, APO, NERICA-L and MAS-946-1 were selected for isolating the endophytic bacterial population from the root tissue. A total of fourteen endophytic isolates were purified from the root tissue of these four varieties. Maximum number of isolates *i.e.* seven were derived from the root tissue of the rice variety APO. All the isolates were designated with the strain name as shown in table 1. The 16S rDNA gene was amplified (Fig. 1) and further sequence analysis of the isolates revealed that all the fourteen isolates were showing 100% homology to different *Bacillus* sp.. These sequences were submitted to NCBI and obtained Gene bank accession numbers (Table 2).

Endophytic bacteria have been reported from various parts of rice plants. Over 95% of the bacteria exist in the plant roots and those plants obtain many nutrients through the soil bacteria (Sang *et al.*, 2014). Plant growth-promoting rhizobacteria genera: *Bacillus* (Idriss *et al.*, 2002), *Enterobacter* (Gupta *et al.*, 1998) and *Corynebacterium* (El Banana and Winkelmann, 1988) have been reported to benefit plants by enhancing plant growth and improving plant health through various direct and indirect mechanisms.

Table 1: Plant growth promoting traits of bacterial isolates.

Rice variety	Strain Name	P-Solubilization	Zn-Solubilization	Siderophore production	HCN production
MAS-946-1	MKA3	-	-	+	-
Nagina-22	MKA9	-	-	-	-
Nagina-22	MKA10	+	-	-	-
Nagina-22	MKA11	+	-	-	-
Nagina-22	MKA13	-	-	-	-
Nagina-22	MKA12	-	-	+	-
APO	MKA14	-	-	-	-
APO	MKA5	-	-	-	-
APO	MKA6	-	-	-	-
APO	MKA1	-	-	-	-
APO	MKA7	-	-	+	-
APO	MKA8	-	-	-	-
APO	MKA2	-	-	-	-
Nerica-L	MKA4	-	-	-	-

Characterization of Plant growth promoting traits of the endophytic isolates

Phosphate solubilisation: Phosphorous availability in the soil is quite high but remains unavailable for plant uptake due to its complex form. Phosphate solubilising bacteria (PSB) as inoculants is an environmental friendly and sustainable approach in meeting phosphate demand of the crops (Mehta and Nautiyal, 2001). It has been reported that bacteria belonging to genera *Bacillus*, *Pseudomonas*, *Serratia*, *Enterobacter* and so on are able to

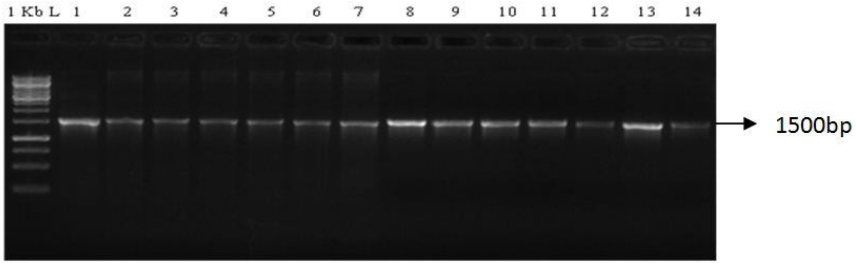

Fig. 1: 16S rDNA gene amplification. Lane: 1-MKA1, 2-MKA2, 3- MKA3, 4- MKA4, 5- MKA5, 6- MKA6, 7- MKA7, 8- MKA8, 9- MKA9, 10- MKA10, 11- MKA11, 12- MKA12, 13- MKA13, 14- MKA14

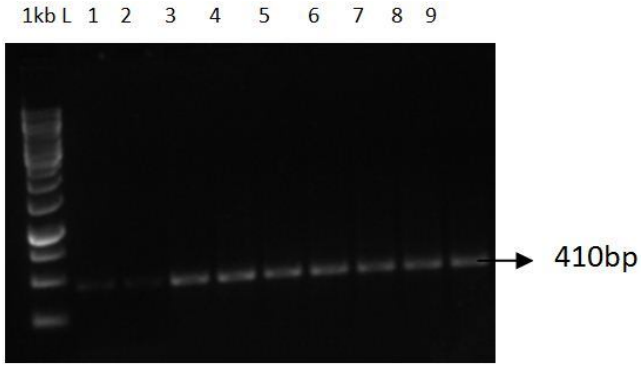

Fig. 2: *nifH* gene amplification. Lane: 1-MKA1, 2-MKA2, 3- MKA3, 4- MKA4, 5- MKA5, 6- MKA6, 7- MKA7, 8- MKA10, 9- MKA14.

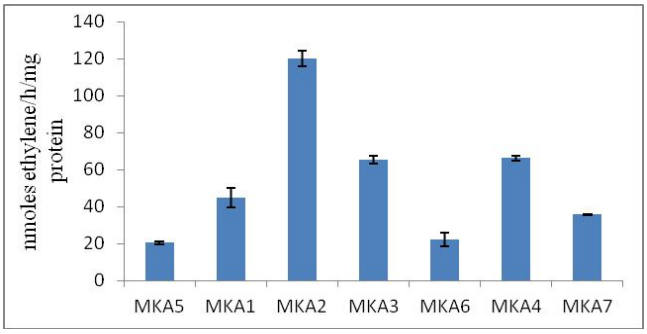

Fig. 3: Graph showing the nitrogenase enzyme activity (nmoles ethylene/h/mg protein) of the seven isolates

solubilize the insoluble phosphate compounds and aid in plant growth (Frey-Klett *et al.*, 2005; Hameeda *et al.*, 2008). Here the isolates were screened for their phosphate solubilising ability. Formation of the halo zone around the isolates were considered to be as phosphate solubilising bacteria. Among the fourteen isolates tested, two of the isolates MKA10 & MKA11 were showing the formation of halo zones and thus having phosphate solubilisation ability in them (Table 1, Fig. 4D). Thus these two isolates have the potential to be used as commercial phosphate solubilising biofertilizer.

IAA production

IAA is the main auxin in plants, controlling many important physiological processes including cell enlargement and division, tissue differentiation and responses to light (Frey-Klett *et al.*, 2005; Gordan and Weber, 1950; Khalid *et al.*, 2004; Leveau and Lindow, 2005). Isolates were screened for IAA production. Eight of the isolates showed IAA production in the range of 4.7±0.15 to 25±0.83 µg/ml (Table 3, Fig. 4A). Maximum amount of IAA was produced by the isolate MKA3. Thus the isolate MKA3 can be used in increasing growth of the other crop plants through the enhancement of IAA production.

Siderophore production

Earlier reports confirmed that bacteria producing siderophore significantly influenced the uptake of several metals including Fe, Zn and Cu by plants (Carrillo-Castaneda *et al.*, 2005; Egamberdiyeva, 2007; Dimkpa *et al.*, 2008; Dimkpa *et al.*, 2009; Gururani *et al.*, 2012). Siderophore also plays an important role in determining the competitive fitness of bacteria to colonize plant roots and to compete for iron with other microorganisms in the rhizosphere. Among the fourteen isolates tested three of the isolates *i.e.* MKA3, MKA12 and MKA7 were confirmed to produce siderophore as shown in table 1, fig. 4B.

Zinc solubilisation

Zinc although a micronutrient, is quite crucial for the development of plants. It is mostly available in complex form in soil. In crops zinc deficiency is one of the most common deficiency worldwide. Bacteria solubilize zinc and make it available to plants for uptake through various mechanisms. According to previous reports, *Bacillus*

Table 2: Identification of isolates by 16S rDNA sequence analysis.

Strain name	Homologous microorganism (% Identity)	Genebank Accession ID
MKA1	*Bacillus* sp. (100%)	MK245986
MKA2	*Bacillus lentus*(100%)	MH552996
MKA3	*Bacillus subtilis*(100%)	MH552997
MKA4	*Bacillus cereus* (100%)	MH552998
MKA5	*Bacillus* sp. (100%)	MK245995
MKA6	*Bacillus* sp. (100%)	MK245987
MKA7	*Bacillus subtilis*(100%)	MK245988
MKA8	*Bacillus aryabhattai*(100%)	MK245994
MKA9	*Bacillus* sp. (100%)	MK245989
MKA10	*Bacillus altitudinis*(100%)	MK245990
MKA11	*Paenibacillus* sp. (100%)	MK245991
MKA12	*Bacillus* sp. (100%)	MK245993
MKA13	*Bacillus licheniformis*(100%)	MK245992
MKA14	*Bacillus pseudomycoides*(100%)	MK245985

strains solubilize unavailable zinc through secretion of organic acids, production of chelating ligands, amino acid, vitamins and phytohormones (Wakatsuki, 1995; Saravanan *et al.*, 2007).

Isolates here were also tested for their Zn solubilising ability. None of the isolates tested positive for Zn solubilisation ability (Table 1).

HCN production

HCN plays an important role in biological control of pathogens (Haas and Défago, 2005). Among all the tested isolates none of the isolate showed HCN production (Table 1).

Screening of diazotrophs

In order to determine the nitrogen fixation, the isolates were first screened in Nitrogen free agar media by doing three subsequent sub culturing. Seven of the isolates were showing growth on nitrogen free media. Amplification of *nifH* gene was also carried out. Nine of the isolates were showing *nifH* gene amplification (Fig. 2) which was further confirmed by sequencing. Sequences were submitted to NCBI (Table 4). To check the activity of the nitrogenase enzyme in all the isolates Acetylene reduction assay was done. Seven isolates tested positive for ARA activity and were showing nitrogenase activity in the range of 20-120 nmoles ethylene produced/h/mg protein (Fig. 3). Although nine isolates were showing *nifH* gene amplification but only seven isolates were having an active nitrogenase enzyme in them.

Earlier reports show that as many as 20 types of endophytic bacteria, comprising both nitrogen-fixing and non-nitrogen- fixing from roots and shoots of rice plants have been isolated (Ladha and Reddy, 2000). Higher proportion of diazotrophic community (Modi *et al.*, 2017) in roots compared with shoots supports the presumption that roots may be a prime niche for growth and nitrogen fixation by endophytes (James and Olivares, 1998; Ladha and Reddy, 2000).

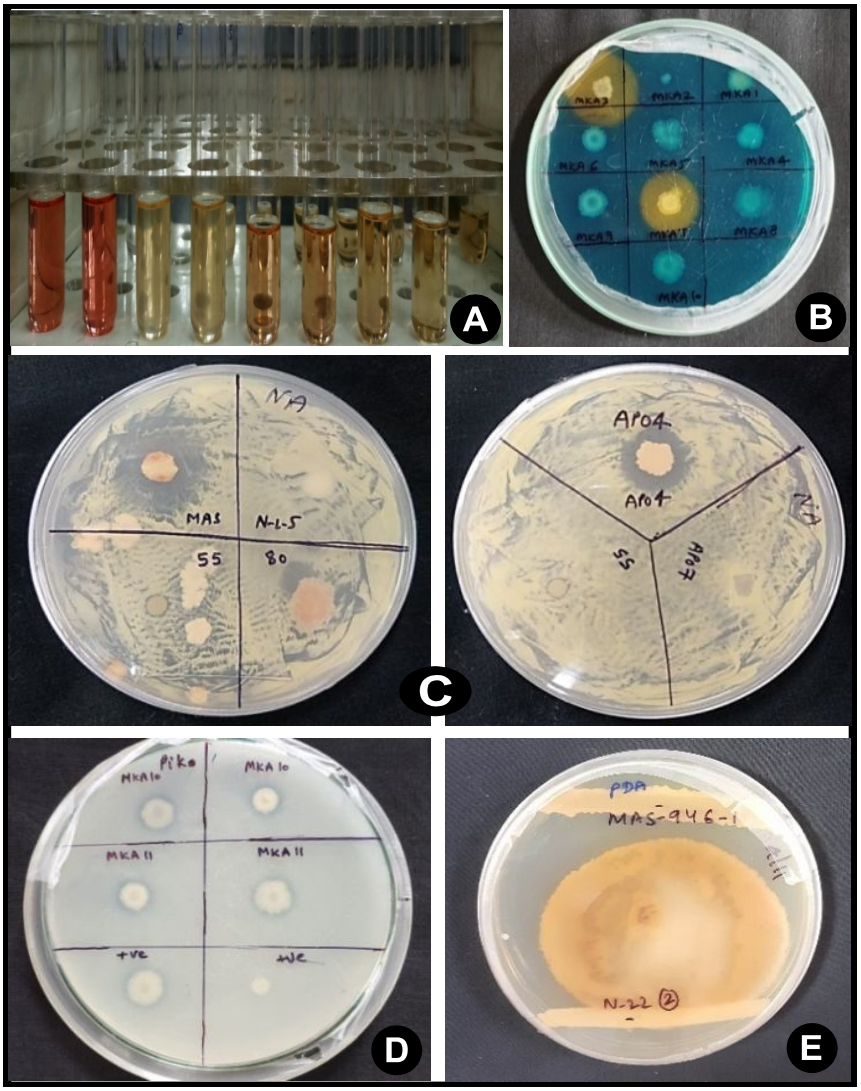

Fig. 4: (A) IAA production, (B) Siderophore production, (C) Antagonistic activity against *Xanthomonas oryzae* pv. *oryzae*, (D) Phosphate solubilisation by the isolates, (E) Antagonistic activity against fungus *Rhizoctonia solani.*

Antagonistic assays

The isolates were also screened for antagonism against *Xanthomonas oryzae* pv. *oryzae.* Results indicate that two of the isolates MKA1 and MKA3 showed the formation of zone of inhibition and thus antagonistic behavior against *Xanthomonas oryzae* pv. *oryzae* (Fig. 4C).

Xanthomonas oryzae pv. *oryzae* is a bacterial pathovar which causes serious blight of rice. Thus antagonistic screening of the isolates is an essential step for preliminary assessment to identify potential isolates that produce antibacterial effect towards the pathogen.

Screening of the isolates for their biocontrol activity against *Rhizoctonia solani AG1* was carried out. One of the isolates MKA3 inhibited the growth of the fungus as revealed by dual culture method (Fig. 4E). Thus this bacterium might be possessing bacterial mycophagy *i.e.* feeding on fungi as source of nutrients and thus inhibiting its growth. Such bacterium can be used as biocontrol agents to prevent from the devastating effects of the fungus *Rhizoctonia solani.*

Table 3: Isolates showing IAA production (µg/ml).

Isolate	-Tryptophan (µg/ml)	+Tryptophan (µg/ml)
MKA1	5.77±0.91	12.60±0.26
MKA2	8.09±0.95	16.48±0.83
MKA3	12.19±0.13	25.018±0.83
MKA4	5.53±0.02	12±0.26
MKA6	5.6±0.47	13.65±0.12
MKA8	6.43±0.59	15.57±0.37
MKA9	4.71±0.16	12.28±0.35
MKA12	7.1±0.43	16.23±0.36

Table 4: Accession ID as provided by NCBI for the *nifH* sequences submitted.

Strain Name	Genebank Accession ID
MKA1	MN364702
MKA2	MN364704
MKA3	MN364696
MKA4	MN364697
MKA5	MN364699
MKA6	MN364700
MKA7	MN364701
MKA10	MN364703
MKA14	MN364698

In sustainable agriculture, certain plant pathogens can be controlled by biological agents like plant growth promoting bacteria (PGPB) and at the same time, PGPB was used as bio-fertilizer (Yasmin *et al.*, 2009). There are a lot of PGPB strains that are reported to suppress numerous of plant pathogen, reduce the disease incidence, stimulate the plant growth factor and supplies the nutrition for the growth of plant (Yasmin *et al.*, 2009; Chithrashree *et al.*, 2011; Hariprasad *et al.*, 2009). Therefore, it has been a considerable research interest in the potential use of antagonistic bacteria as PGPB (Babalola, 2010; Yasmin *et al.*, 2009).

Conclusion

A total of fourteen endophytic bacteria, identified to be different *Bacillus* sp. were screened here for different PGP traits. Our results revealed that two isolates showed phosphate solubilising activity, three isolates showed siderophore producing activity, eight isolates showed auxin production, seven isolates showed active nitrogenase enzyme, two of the isolates showed antagonistic activity against *Xanthomonas oryzae* pv. *oryzae* and one against the fungus *Rhizoctonia solani AG1*. These isolates having plant growth promoting properties can be further checked to see their effect on plant growth promotion by doing bioassays. Among all the isolates MKA3 which was identified to be *Bacillus subtilis* was found to be the most potent strain in displaying many PGP traits that makes it a possible candidate for being used as a biofertilizer.

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References

Babalola, O.O. (2010). Beneficial bacteria of agricultural importance. *Biotech Lett*., **32:** 1559-70.

Bhattacharyya, P.N. and D.K. Jha (2012). Plant growth-promoting rhizobacteria (PGPR):Emergence in agriculture. *World J. Microbiol. Biotechnol*., **28:** 1327-1350.

Carrillo-Castaneda, G., J.J. Munoz, J.R. Peralta-Videa, E. Gomez and J.L. Gardea-Torresdey (2005). Modulation of uptake and translocation of iron and copper from root to shoot in common bean by siderophore-producing microorganisms. *J. Plant Nutr.,* **28:** 1853-65.

Castric, P.A. (1975). Hydrogen cyanide, asecondary metabolite of *Pseudomonas aeruginosa*. *Can. J. Microbiol.*, **21:** 613-618.

Chithrashree, A.C. Udayashankar, S. Chandra Nayaka, M.S. Reddy and C. Srinivas (2011). Plant growth-promoting rhizobacteria mediate induced systemic resistance in rice against bacterial leaf blight caused by *Xanthomonas oryzae* pv *oryzae*. *Biol. Control*., **59:** 114-22.

Dimkpa, C., A. Svatos, D. Merten, G. Buchel and E. Kothe (2008). Hydroxamatesiderophores produced by *Streptomyces acidiscabies* E13 bind nickel and promote growth in cowpea (*Vigna unguiculata* L.) under nickel stress. *Can. J. Microbiol*., **54:** 163-72.

Dimkpa, C., D. Merten, A. Svato˘s, G. B¨uchel and E. Kothe (2009). Siderophores mediate reduced and increased uptake of cadmium by *Streptomyces tendae* F4 and sunflower (*Helianthus annuus*), respectively. *J. Appl. Microbiol.,* **107:** 1687-96.

East, R. (2013). Microbiome: Soil science comes to life. *Nature.,* **501:** S18-S19.

Egamberdiyeva, D. (2007). The effect of plant growth promoting bacteria on growth and nutrient uptake of maize in two different soils. *Appl. Soil. Ecol.*, **36:** 184-9.

El-Banana, N. and G. Winkelmann (1988). Pyrrolnitrin from *Burkholderiacepacia*: antibiotic activity against fungi and novel activities against streptomycetes. *J. Appl. Microbiol.*, **85:** 69-78.

Food and Agriculture Organization of the United Nations (2013). FAO Statistical Yearbook, World Food and Agriculture; FAO: Rome, Italy, 307.

Frey-Klett, P., M. Chavatte, M.L. Clausse, S. Courrier, C.L. Roux and J. Raaijmakers (2005). Ecto-mycorrhizal symbiosis affects functional diversity of rhizosphere fluorescent pseudomonads. *New Phytol.*, **165:** 317-28.

Gordon, S.A. and R.P. Weber(1950). Colorimetric estimation of indole acetic acid. *Plant Physiol.*, **26:** 192-5.

Gupta, A., A.K. Saxena, M. Gopal and K.V.B.R. Tilak (1998). Effect of plant growth promoting rhizo bacteria on competitive ability of introduced Brady rhizobium sp. (Vigna) for nodulation. *Microbiol Res*., **153:**113-7.

Gururani, M.A., C.P. Upadhyaya, V. Baskar, J. Venkatesh, A Nookaraju and S.W. Park (2012). Plant growth-promoting rhizobacteria enhance abiotic stress tolerance in *Solanum tuberosum* through inducing changes in the expression of ROS-scavenging enzymes and improved photosynthetic performance. *J. Plant Growth Regul.*, **32:** 245-258.

Haas, D. and G. Défago (2005). "Biological control of soil-borne pathogens by fluorescent pseudomonads," *Nature Reviews Microbiology.*, **3(4):** 307-319.

Hameeda, B., G. Harini, O.P. Rupela, S.P. Wani and G. Reddy (2008). Growth promotion of maize by phosphate-solubilizing bacteria isolated from composts and macrofauna. *Microbiol Res.*, **163:** 234-42.

Hariprasad, P., H.M. Navya, S. Chandra Nayaka and S.R. Niranjana (2009). Advantage of using PSIRB over PSRB and IRB to improve plant health of tomato. *Biol. Control.*, **50:** 307-16.

Idriss, E.E., O. Makarewicz, A. Farouk, K. Rosner, R. Greiner and H. Bochow (2002). Extra-cellular phytase activity of *Bacillus amyloiquefaciens* FZB45 contributes to its plant-growth-promoting effect. *Microbiology.*, **148:** 2097-109.

James, E.K. and F.L. Olivares (1998). Infection and colonization of sugar cane and other graminaceous plants by endophyticdiazotrophs. *Critical Reviews in Plant Sciences*., **17:** 77-119.

Khalid, A., M. Arshad and Z.A. Zahir (2004). Screening plant growth-promoting rhizobacteria for improving growth and yield of wheat. *J. Appl. Microbiol.,* **96:** 473-80.

Ladha, J.K. and P.M. Reddy (2000). The quest for nitrogen fixation in rice. Proceedings of the Third Working Group Meeting on Assessing Opportunities for Nitrogen Fixation in Rice. 9-12 Aug. 1999, IRRI, Los Banos, Laguna, Philippines, 354.

Lee, K.K. and T. Yoshida (1997). An assay technique of measurement of nitrogenase activity in root zone of rice for varietal screening by the acetylene reduction method. *P. Soil.*, **46:** 127-134.

Leveau, H.J. and S.E. Lindow (2005). Utilization of the plant hormone indole-3-acetic acid for growth by *Pseudomonas putida* strain, 1290, *J. Appl. Microbiol.*, **71:** 2365-71.

Luo, X.S., X.Q. Fu, Y. Yang, P. Cai, S.B. Peng and W.L. Chen (2016). Microbial communities play important roles in modulating paddy soil fertility. *Sci. Rep.*, **6:** 20326.

Mehta, S. and S.C. Nautiyal (2001). An efficient method for qualitative screening of phosphate-solubilizing bacteria. *Cur. Microbiol.*, **43:** 51-6.

Milagres, A.M.F., A. Machuca and D. Napoleão (1999). Detection of siderophore production from several fungi and bacteria by a modification of chromeazurol S (CAS) agar plate assay. *J. Microbiol. M.,* **37(1):** 1-6.

Modi, K., P. Patel and K. Parmar (2017). Isolation, Screening and Characterization of PGPR from Rhizosphere of Rice. *Int. J. Pure App. Biosci*., **5(3):** 264-270.

Moronta-Barrios, F., F. Gionechetti, A. Pallavicini, E. Marys, V. Venturi, F. Moronta-Barrios, F. Gionechetti, A. Pallavicini, E. Marys and V. Venturi (2018). Bacterial Microbiota of Rice Roots: 16S-Based Taxonomic Profiling of Endophytic and Rhizospheric Diversity, Endophytes Isolation and Simplified Endophytic Community. *Microorganisms*, **6:** 14.

Naher, U.A., R. Othman, Q.A. Panhwar and M.R. Ismail (2015). Biofertilizer for sustainable rice production and reduction of environmental pollution. In Crop Production and Global Environmental Issues; Springer: Berlin, Germany, 283-291.

Patten, C.L. and B.R. Glick (1996). Bacterial biosynthesis of indole-3-acetic acid. *Can. J. Microbiol*., **42:** 207-220.

Pikovskaya, R.I. (1948). Mobilization of phosphorous in soil in connection with vital activity of some microbial species. *Mikrobiologiya.,***17:** 362-370.

Sakthivel, N. and S.S. Gnanamanickam (1986). Toxicity of *Pseudomonas fluorescens* towards rice sheath rot pathogen *Acrocylindrium oryzae*. *Curr. Sci.*, **5:** 106-107.

Sang, Hye, Ji, Mayank, Anand, Gururani and Se-Chul, Chun (2014). Isolation and characterization of plant growth promoting endophyticdiazo trophic bacteria from Korean rice cultivars. *Microbiological Research.*, **169:** 83-98.

Santoyo, G., G. Moreno-Hagelsieb, M. del Carmen Orozco-Mosqueda and B.R. Glick (2016). Plant growth-promoting bacterial endophytes. *Microbiol. Res*., **183:** 92-99.

Saravanan, V.S., M. Madhaiyan and M. Thangaraju (2007). Solubilization of zinc compounds by the diazotrophic, plant growth promoting bacterium *Gluconacetobacter diazotrophicus*. *Chemosphere.*, **66:** 1794-1798.

Sharti, A.T. and R.R. Vittal (2013). Biocontrol potentials of plant growth promoting rhizobacteria against Fusarium wilt disease of cucurbit. *ESci. J. Plant Pathol.*, **02(03):** 155-61.

Singh, J.S., V.C. Pandey and D.P. Singh (2011). Efficient soil microorganisms: A new dimension for sustainable agriculture and environmental development. *Agric. Ecosyst. Environ*., **140:** 339-353.

Vijay, K.K.K., S.K.R. Yellareddygari, M.S. Reddy, J.W. Kloepper, K.S. Lawrence, X.G. Zhou, H. Sudini, D.E. Groth, S. KrishnamRaju and M.E. Miller (2012). Efficacy of *Bacillus subtilis* MBI 600 against sheath blight caused by *Rhizoctonia solani* and on growth and yield of rice. *Rice Sci*., **19:** 55-63.

V. Sandhya, M., S.K. Shrivastava, Z. Ali. and V. Sai Shiva Krishna Prasad (2017). Endophytes from maize with plant growth promotion and biocontrol activity under drought stress. *Russian Agricultural Sciences.*, **43(1):** 22.

Wakatsuki, T. (1995). Metal oxidoreduction by microbial cells. *J. Ind. Microbiol*., **14:** 169-177.

Yasmin, F., R. Othman, K. Sijam and M.S. Saad (2009). Characterization of beneficial properties of plant growth-promoting rhizobacteria isolated from sweet potato rhizosphere. *Afr. J. Microbiol. Res*., **3(11):** 815-21.