Oryza Vol. 54 No. 2, 2017 (208-219)
DOI 10.5958/2249-5266.2017.00027.3

Functional characterization of phosphate solubilizing bacteria of coastal rice soils of Odisha, India

Nilima Dash, Avishek Pahari and TK Dangar*

ICAR-National Rice Research Institute, Cuttack 753006, Odisha, India

****Corresponding author e-mail: dangartk@gmail.com***

Received : 12 June 2017 Accepted : 11 July 2017 Published : 28 September 2017

ABSTRACT

In soil, different plant growth-promoting bacteria (PGPB) convert insoluble phosphorus to available forms for plants which helps to enhance crop productivity. Inoculation of phosphate solubilizing bacteria (PSB) as biofertilizer enhances P availability to plants. Therefore, microbial dynamics of coastal rice field soils of Balasore (Chandipur, Talapada and Talasari) and Cuttack (NRRI), Odisha were analyzed, and PSB were isolated, characterized and assessed for plant growth promotion (PGP) functions. The PSB populations in the soils of Chandipur, Talapada, Talasari and Cuttack (NRRI) were 0.2, 2.5, 2.8 and 3.6 (×10 ^{5}) cfu/g soil, respectively. Sixteen bacterial isolates were assessed qualitatively for phosphate solubilization, out of which, six higher efficient organisms (phenotyped as Bacillus spp.) possessing polyvalent PGP functions viz. nitrogen fixation; IAA, ammonia and siderophore production, and phosphate mineralization were studied thoroughly. The six PSB possessing the beneficial PGP traits can be exploited as potent biofertilizers to sustain rice production.

Key words: *Phosphate solubilization, plant growth promotion, Bacillus, rice*

INTRODUCTION

Phosphorus is the second most important nutrient next to nitrogen for growth, development, structural and metabolic functions of plants and comprises about 0.2% plant dry weight. It is a component of DNA, RNA, ATP, phospholipids of cell membranes, a major chemical energy repository required for metabolism, and promotion of N_2 fixation in legumes (Saber et al., 2005). Soil phosphorus accounts to about 0.05%, out of which only 0.1% is available to the plants. Moreover the phosphate anions react with cations *viz.*, Ca^{2+}, Mg^{2+}, Fe^{3+} and Al^{3+} depending on the soil properties. The cation bound forms of P are highly insoluble and it becomes unavailable to plants which reduces overall P use efficiency even after phosphatic fertilizer application (Vassilev and Vassileva, 2003). To sustain crop production, soluble forms of inorganic P is applied frequently which leaches to the surface and ground water bodies causing eutrophication of aquatic environments (Smyth et al., 2011). The phosphate solubilizing (PS) microbes (PSM) mineralize phosphate which reduces P deficiency in soil, increases soluble phosphate availability which enhances plant growth, biological nitrogen fixation (BNF) efficiency of diazotrophs, plant growth promotion regulators (PGR) and availability of trace elements like iron, zinc, copper, boron, manganese, molybdenum etc. (Saber et al., 2005; Ponmurugan and Gopi, 2006). Therefore, rice rhizospheric PSB were assessed to select and characterize more potent polyvalent P solubilizing plant growth promoting bacteria (PGPB) intended to improve/ sustain rice production in P deficient soils and reduce phosphatic fertilizer requirement.

MATERIALS AND METHODS

Collection and physical properties of soil samples

Soil samples were collected aseptically from medium-land rice fields of Cuttack (NRRI) and Balasore (Talasari, Talapada and Chandipur) of Odisha. About 1

cm top soil was removed, 10 g samples were collected from five spots of each location and mixed thoroughly. The soils were air-dried up to 20% moisture level, powdered, passed through a 200 mesh sieve and stored in polybags within desiccators at RT. Moisture level of the soils were maintained at 20% level and, soil type, pH, Eh, total organic C (TOC), available K (AK) and available P (AP) contents were analyzed.

Isolation of phosphate solubilizing bacteria from soil samples

In 10 ml sterile distilled water, one g soil was suspended, logarithmic dilutions were made up to 10^{-4} level and 100 µl of each suspension was added seprately with 100 ml National Botanical Research Institute phosphate (NBRIP) medium, plated in four plates and incubated at 30±0.1^{0}C for 72 h. The organisms which formed a clear zone around the colonies were isolated (n=16). The isolates were subcultured and preserved on NA slants at 40±1^{0}C.

Qualitative and quantitative estimation of phosphate solubilization

The isolates were spotted on 3 replicated plates of NBRIP medium. The plates were incubated for 3d at 30 ± 0.1ºC. P solubilization efficiency was estimated from the ratio of the diameter of clear zones around the colonies and the bacterial colonies, and expressed as P solubilization index (PSI) following the formula (Premono et al., 1996; Ponmurugan and Gopi, 2006): PSI=(Z+C)/C, where, Z is diameter of clearing zone and C is diameter of colony.

Inorganic phosphate ($Ca_3(PO_4)_2$) solubilization in broth culture was estimated quantitatively after growing the organisms in triplicate Erlenmeyer flasks (250 ml) containing 100 ml NBRIP broth. Uninoculated medium was used as control. The flasks were incubated for 1 to 3d at 30 ± 1°C on a rotary shaker at 85 rpm. The supernatants were collected after centrifugation of the broth culture at 10000 rpm for 10 min. Sulfo-molybdate reagent was used to estimate the phosphorus content in culture supernatant (Olsen et al., 1954). To 1 ml culture supernatant, 2 ml each of 2.5% sulfomolybdate solution, 0.25% p-nitrophenol indicator, 1N H_2SO_4 and 1 ml stannous chloride solution (40% w/v in HCl) were added, and the absorption was recorded at 660 nm. P solubilization was expressed in terms of µg/ml phosphorus released in culture medium.

Assessment of plant growth promoting traits

To test indole production, the isolates were grown in broth medium (g/l): peptone 20, NaCl 5 and tryptophan 0.1%, pH 7) and incubated at 30±0.1 °C. To the broth, 1 ml Salkowski's reagent (50 ml 35% $HClO_4$ containing 0.5M $FeCl_3$) was added, mixed and change of medium to pink colour indicated indole production (Bric et al., 1991). Chromeazurol S (CAS) assay was used to detect siderophore production. The isolates were spot inoculated on the CAS agar medium composed of solution A (chromazurol 60.6 mg dissolved in 50 ml water), 10 ml solution B (1 mM $FeCl_3.H_2O$ in 10 mM HCl), solution C (CTAB 72.9 mg dissolved in 40 ml water) and 300 ml nutrient agar), incubated at 30±0.1 °C for 5-6 d. Siderophore production was revealed by yellow to orange zone formation against blue background (Schwyn and Neilands, 1987). To assess ammonia production, the isolates were inoculated in 5 ml peptone water (g/l: peptone 10, NaCl 5) and incubated for 96 h at 30±0.1°C. Development of brown colour on addition of Nessler's reagent (70.83 g/l K_2HgI_4 in 2.5 M KOH) indicated ammonia production. Hydrogen cyanide (HCN) production by the bacteria was carried out qualitatively. The isolates were inoculated in the medium (g/l: proteose peptone 20, glycerol 10 ml, $MgSO_4$ 1.5, K_2HPO_4 1.5, glycine 4.4, pH 7). Filter paper strips (0.5 cm wide) soaked in picric acid reagent (0.5 g picric acid and 2 g Na_2CO_3 dissolved in 100 ml distilled water) were plugged inside the rim of the flask and incubated at 30 ± 0.2°C for five days. Change of colour of filter papers from yellow to brown indicated HCN production (Bakker and Schippers, 1987). The PSB (n = 6) exhibiting optimum PGP traits were thoroughly characterized.

Cultural characterization of the phosphate solubilizing bacteria

Cultural (colony), and morphological, physiological and biochemical characters of the 6 selected bacteria, growth of the organisms on nutrient agar (NA) medium (g/l: peptone 5, sodium chloride 3, beef extract 3, agar 18, pH 7.0) supplemented with 1 to 15% NaCl, extracellular enzyme production, response of the organism to different antibiotics were studied following standard methods and identified the isolates (Collee and

Miles, 1989; Smibert and Krieg, 1995; Lacey, 1997; Logan et al., 2009; Krieg, 2015). The turbidity were plotted against time function to determine the growth kinetics.

Analysis of organic constituents of the bacteria

Sugar, amino acid and protein were extracted using standard methods (Reed et al., 1984). The bacteria were grown in nutrient broth, the bacterial pellets were collected through centrifugation at 7000 rpm at 30± 0.1^0C, washed with sterile distilled water, re-centrifuged, pellet resuspended in 1 ml water and 100 µl each suspension was taken for dry weight measurement. The remainder suspensions were further centrifuged at 7000 rpm, 30± 0.1^0C, the pellets were taken; 5 ml 80% ethanol was added to each pellet, boiled for 5 min, cooled to room temperature and stored overnight at 4°C. The suspensions were centrifuged; the supernatants and pellets were allowed for evaporation of alcohol. After evaporation of each supernatant, 2 ml water was added and mixed thoroughly. To 1 ml of each extract, 4 ml anthrone reagent (0.2 % anthrone in conc. H_2SO_4) was added and boiled in a water bath at 100^0C for 10 min covering the mouth with plastic caps to avoid evaporation, cooled to room temperature and OD was taken at 625 nm through a spectrophotometer. Amount of sugar was calculated as glucose equivalent from the standard curve. To the remainder 1 ml of each supernatant, 1 ml ninhydrin reagent (80 mg $SnCl_2$ in 50 ml citrate buffer mixed with 2 g ninhydrin in 50 ml ethylene glycol monomethyl ether) were added, boiled for 20 min, 5 ml diluting solution (equal volume mixture of water and n-propanol) was added and OD was taken at 570 nm. Amino acid content was estimated as glycine equivalent from the standard curve. The dry pellets were crushed with 1-2 ml 0.3N KOH separately, stored overnight at 37 ± 0.1^0C, centrifuged (8000 rpm, 4 ± 0.1^0C, 5 min) and the supernatants were collected. To 100 µl of each supernatant and blank (100 µl 0.3N KOH), 5 ml commercial Bradford reagent was added and OD was taken at 595 nm. Amount of protein was calculated from the standard curve of BSA.

Molecular analysis of the isolates

Genomic DNA was extracted using Himedia Genomic DNA kit. Plasmid was isolated following Jensen et al. (1995). Cells or membrane fractions were resuspended in TBS buffer, and then extracted with TritonX114 incubated on an ice bath. The mixture was warmed and separated into two protein containing phases. The upper phase contained hydrophilic proteins and the lower phase contained ampiphillic proteins. Both phases were analyzed through gel electrophoresis to profile proteins. Agarose gel (1%) electrophoresis was performed for the plasmid and genomic DNA, cellular proteins were analyzed by SDS PAGE (6%) and the isozymes of amylase and catalase enzymes were assessed by native PAGE (8%) (Thimmaiah, 1999). The 16S rDNA primers (27F-AGAGTTTGATCCTGGCTCAG and 1495R-AAGGAGG TGATCCAGCCGCA) were used for amplification using genomic DNA template following the PCR cycling conditions: denaturation at 95°C for 5 min, followed by 95°C for 30 sec, 55°C for 30 sec, 72°C for 2 min for 30 cycles and final extension at 72°C for 10 min and 4°C for 10 min. Restriction digestion profile of amplified 16S rDNA of the isolates was studied using 3 enzymes (*BamHI*, *EcoRI* and *HaeIII*). PCR amplification for pqq genes (*pqq*, *pqqAB12*, *pqqCF34*) was carried out using the genomic DNA of the isolates. PCR cycling protocol for pqq genes were 95°C for 5 min once, followed by 95°C for 30 sec, 54°C for 30 sec, 72°C for 2 min for 30 cycles and final extension at 72°C for 10 min and 4°C for 10 min.

RESULTS AND DISCUSSION

Physico-chemical properties of rice field soils

The soil physico-chemical properties of NRRI differed from 3 soils of Balasore (Table 1). The NRRI rice field

Table 1. Soil characters and P-solubilizing bacterial population in different soils

Soil no.	Location	Site	Soil type	pH	Eh(dS/m)	Total organic C (%)	Available P (kg/ha)	Available K (kg/ha)	P solubilizer (cfu/g soil)
144	Chandipur, Balasore	Paddy field	Sandy	5.03	2.80	0.40	33.00	35.20	2.0×10^4
145	Talapada, Balasore	Paddy field	Sandy	4.72	5.40	0.38	27.00	24.40	2.5×10^5
146	Talasari, Balasore	Paddy field	Sandy	5.72	1.68	0.26	33.00	17.80	2.8×10^5
149	NRRI, Cuttack	Paddy field	Sandy loam	6.53	5.32	0.45	22.20	102.06	3.6×10^5

Table 2. PGP functions of the bacteria

Test	Isolate number					
	AP2	AP6	AP7	AP8	AP9	AP12
Siderephore production (µg/g dr. wt.)	+	-	-	-	+	+
	0.011	-	-	-	0.011	0.015
HCN production	-	-	-	-	-	-
NH3 production	+W	-	+	+W	+W	+W
IAA production	-	-	-	-	-	-
N2 fixation	+	+	+	+	+	+
Phosphate solubilization (µg/ml)*	+, 1.04#	+, 1.11#	+, 1.10#	+, 1.33#	+, 1.18#	+, 1.15#
	68.23±0.22	198.70±0.76	142.69±0.47	402.51±0.47	293.70±0.35	184.47±0.15

*Results are mean of three replications ±SE. #PSI. The PSB *viz.*, AP 3, 5, 10, 13 and 16 also produced siderophore, but 5 organisms *viz.*, AP 1, 4, 11, 14 and 15 had no other PGP functions.

has sandy loam (sandy in Balasore fields) soil; higher pH (6.5), Eh (5.32 dS/m), TOC (0.45%) and AK (102.06 kg/ha) but lower AP (22.02 kg/ha) levels thanBalasore (Table 1).

Population dynamics and isolation of phosphate solubilizing bacteria from different soils

The PSB population in the soils of Chandipur (no. 144), Talapada (no. 145), Talasari (no. 146) and NRRI (no. 149) were 0.2, 2.5, 2.8 and 3.6 ($\times 10^5$), cfu/g, respectively (Table 1) (Figs. 1, 2). From different soils 16 (AP 1-16) PSB (Figs. 1-4) were isolated and assessed.

Efficiency of P solubilization, PGP functions and selection of efficient bacteria

Change of pH and P solubilization in broth by 6 most potent PSB were recorded at 24h intervals for 72 h (Fig. 5). It was observed that AP 6 solubilized maximum P (140.83 µg/g dr. wt.) with decline to pH 4.81 in 1d, but decline to pH 4.17 and maximum P (402 µg/ml) solubilization was recorded for AP 8 after 3 d (Table 2; Fig. 5). None of the isolates produced indole acetic acid but all produced ammonia (except for AP 6), mineralized phosphate, fixed nitrogen (Table 2) and AP 2, AP 3, AP 5, AP 9, AP 10, AP 12, AP 13 and AP 16 produced siderophore (Table 2, Fig. 6). Among them, six isolates *viz.*, AP 2, AP 6, AP 7, AP 8, AP 9 and AP 12 were selected for further studies as they solubilized more phosphate and possessed polyvalent PGP functions like nitrogen fixation, siderophore, IAA, ammonia production (Table 2).

Morphological characteristics of the bacteria

The colonies of the potent polyvalent PSB were irregular, white or off-white, raised or flat, non-gummy, undulate and colony sizes measured 3-6 mm on NA after 3 d (Table 3). Length of the organisms ranged 2.05-3.8 µm and diameter 1.4-2.35 µm, and the cell of the isolate AP 12 was larger than AP 2, AP 7, AP 8 and AP 9 (Table 3; Figs. 2-4). All bacteria were Gram positive and spore forming rods (Fig. 3), spores turned green with malachite green stain and red vegetative cells with safranin (Fig. 4).

Fig. 1. Phosphate solubilization in plate

Fig. 2. Phase contrast photomicrograph

Table 3. Characterization and identification of bacteria

Characters			Isolate no.					
			AP 2	AP 6	AP 7	AP 8	AP 9	AP 12
Colony	Form		Irregular	Irregular	Irregular	Irregular	Irregular	Irregular
	Colour		White	Off-white	Off-white	Off-white	Off-white	Off-white
	Elevation		Raised	Raised	Raised	Raised	Raised	Flat
	Margin		Undulate	Undulate	Undulate	Undulate	Undulate	Undulate
	Size (mm)		5	6	4	3	6	4
	Consistency		Non gummy	Non gummy	Non gummy	Non gummy	Non gummy	Non gummy
Cells	Shape		Rod	Rod	Rod	Rod	Rod	Rod
	Length (µm)*	Range	2-4.2	1-3.1	2.2-4.1	1.3-3.1	2-3.9	2.8-4.8
		Mean	3.1	2.05	3.15	2.2	2.95	3.8
	Breadth (µm)*	Range	1-1.8	1-3.1	1.1-2.2	1.2-2	0.9-2.1	1.9-2.8
		Mean	1.4	2.05	1.65	1.6	1.5	2.35
	Motility		Motile	Motile	Motile	Motile	Motile	Motile
	Gram stain		+	+	+	+	+	+
	Spore stain		+	+	+	+	+	+
Physiological and biochemical properties	Catalase		-	+W	+	+	+	+
	H_2S production		-	-	-	-	-	-
	Methyl red test		-	+++	++	-	-	-
	Vogues Proskauer (AMC) test		+	-	-	-	-	-
	Nitrate reduction		++	-	-	-	-	-
	Urease production		-	-	+++	+++	+++	+++
	Citrate utilization		-	-	+	-	-	-
	Oxidase		-	+	+	+	+	+
	Arginine dihydrolase		-	-	-	-	-	-
	Aesculin hydrolysis		+	+	+	+	+	+
Enzymatic activities	Protease	Gelatinase	+	+	+	+	+	+
		Casein hydrolysis	+	+W	+	+	+	+
	Lipase	Tributyrin hydrolysis	+W	+	+	+	+	+
		Tween 80	-	-	-	-	-	-
		Cholesterol hydrolysis	-	-	-	-	-	-
	Amylase		+	+	+	-	+	-
	Lecithinase		-	-	-	-	-	-
	Pectin hydrolysis		+	-	-	-	-	-
	Chitin hydrolysis		-	-	-	-	-	-
	Cellulase		+	+	+	+	+	+
	DNase		-	-	-	-	-	+W
Salt tolerance and growth pH	NA+ NaCl (%)		2	4	14	14	12	10
	pH tolerance		5-10	5-12	5-12	5-10	5-10	5-10
Antibiotic sensitivity (Dose/disc)	Ampicillin (25 µg)		S	S	S	S	S	S
	Ciprofloxacin (10 µg)		S	S	S	S	S	S
	Chloramphenicol (30 µg)		S	S	S	S	S	S
	Erythromycin (15 µg)		S	S	S	S	S	S
	Gentamycin (50 µg)		S	S	S	S	S	S
	Methicillin (10 µg)		R	R	S	S	S	S
	Neomycin (30 µg)		S	S	S	S	S	S
	Nystatin (100 units)		R	R	R	R	S	R
	Nalidixic acid (30 µg)		S	S	S	S	S	S
	Penicillin G (10 units)		S	R	S	S	S	S
	Streptomycin (10 µg)		S	R	S	S	S	S
	Tetracycline (30 µg)		S	S	S	S	S	S
	Rifampicin (5µg)		R	S	S	S	S	S
	Vancomycin (30 µg)		S	S	S	S	S	S
Identification (phenotyping)			*Bacillus cereus*	*B. pantothenticus*	*B. macquariensis*	*B. badius*	*B. badius*	*B. sphaericus*

*Results are means of 3 observations. + = positive result, +w = weakly positive, - = negative result, s= sensitive, r= resistant

Physiological and biochemical characters of the bacteria

All organisms were catalase positive (except for AP 2), VP negative (except for AP 2) and urease positive

Fig. 3. Gram stained bacteria

(except for AP 2 and AP 6). Except for AP 2, AP 8, AP 9 and AP 12, the other organisms were positive for methyl red test, and only AP 7 was citrate test positive (Table 3). The isolates were negative for nitrate reduction (except for AP 2) and hydrolyzed esculin. However, other test results were variable for different organisms. The isolates fermented mannitol, AP 2 produced gas from the carbon compounds (except for arabinose), and acid was produced from fructose by AP 2 only but all of them produced acid from sucrose. All organisms hydrolysed starch (except for AP 8 and AP 12) and tributyrin (but Tween 80) and AP 2 hydrolyzed pectin, but none metabolized cholesterol and chitin.

pH and salt tolerance of the bacteria

The pH tolerance limits of the isolates AP 2, AP 8, AP 9 and AP 12 was 5-10, AP 6 and AP 7 was 5-12, and NaCl tolerance limit of AP 2 was 2%, AP 6 was 4%, AP 12 was 10%, AP9 was 12%, AP 7 and AP 8 was 14% (Table 3).

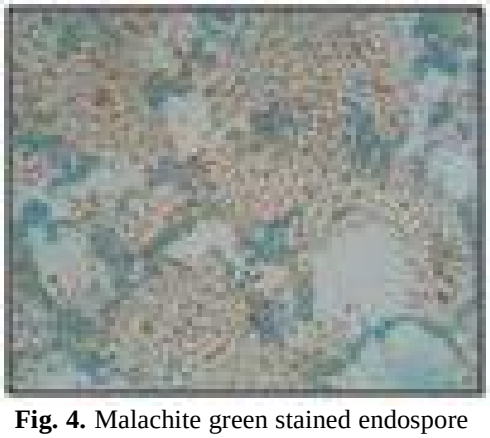

Fig. 4. Malachite green stained endospore

Antibiotic sensitivity of the isolates

All organisms were susceptible to ampicillin, ciprofloxacin, chloramphenicol, erythromycin, gentamycin, neomycin, nalidixic acid, tetracycline and vancomycin, however, AP 6 was resistant to methicillin, penicillin G and streptomycin, AP 2 was resistant to methicillin and rifampicin but only AP 9 was sensitive to nystatin (Table 3).

Growth kinetics of the isolates

The growth kinetics showed that growth phases varied for different organisms *viz.*, AP 8, 9 and 12 had lag period of about 4 h but others had up to 9 h, followed by log (exponential) phases up to 11 h for former organisms but about 17 h for others, the stationary phases ranged between 15-25 h and then the organisms entered into the death phase (Fig. 7).

Organic constituents of the isolates

The bacterium AP 8 contained maximum sugar (237.5 µg/g dr. wt.) and glycine (612.5 µg/g dr. wt.), and AP 6 had minimum sugar (12.3 µg/g dr. wt.) and glycine (58.7 µg/g dr. wt.) but maximum protein (400 µg/g dr. wt.), whereas, AP 2 possessed minimum protein (42.5 µg/g dr. wt.) (Fig. 8).

Genomic DNA, cellular protein, P-solubilizing genes of the isolates

The AP 2 had 4 plasmids of about 6.3-37.9 kbp, AP 9 had 3 plasmids of 8.8-37.9 kbp and AP 12 had 4 plasmids

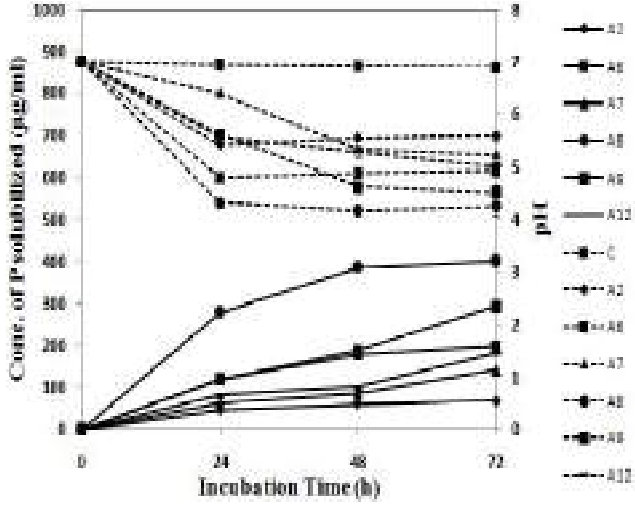

Fig. 5. Kinetics of pH change of NBRIP broth and phosphate solubilization by the bacteria with time

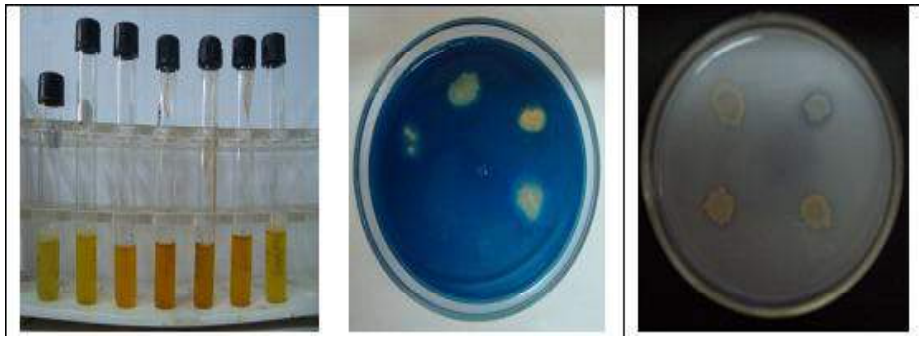

Fig. 6. Qualitative tests of PGP functions of the bacteria

of about 5.8-37.9 kbp sizes (Table 4, Fig. 9). SDS-PAGE profile of the total cellular proteins revealed 15 proteins of 32.2-305.3 kDa for AP 7; 13 proteins of 32.8-305.3 kDa for AP 8 and 10 proteins of 33.1-337.5 kDa sizes for AP 9 (Table 5; Fig. 10). Molecular sizes of the genomic DNA of the isolates were about 14, 14.1, 12.8, 14.3 and 13.2 kDa for AP2, AP7, AP8, AP9 and AP12, respectively (Table 4; Fig. 11) *i.e.*, the size of the genome of the organisms were not similar. *BamHI* had no recognition sites in none of the 16S rDNA (Fig. 12) but *EcoRI* produced 2 prominent fragments of rDNA of AP 7 of approximately 0.91 and 0.71 kbp sizes, AP 8 of about 0.91 and 0.68 kb sizes, AP 9 of about 0.9 and 0.71 kbp sizes and AP 12 of about 0.91 and 0.73 kb sizes, but *HaeIII* produced two prominent fragments of AP 7 (0.64, 0.47 kbp), AP 8 (0.67, 0.5 kbp), AP 9 (0.64, 0.47 kbp) and AP 12 (0.64, 0.48 kbp) sizes (Table 6; Fig. 13). The amylase enzyme of the organisms produced more than 10 isozymic fractions of different sizes spread all over the zymogram (Fig. 14) but they had only one protein fraction for catalase (Fig. 15). The AP 2 possessed the *pqqAB12* gene which produced 170 bp amplicon size in PCR amplification (Fig. 16) and all isolates (except for AP 7) had the pqqCF34 which produced 57 bp amplicon in PCR amplification (Fig. 17).

Numerical abundance of PSB in NRRI soil (3.6 x 10^5 cfu/g) was 1.3 - 18 times more than that (0.2-2.8 x 10^5 cfu/g) in three Balasore soils (Table 1). Nutrition poor sandy soil due to lower TOC (0.26-0.40%) and AK (17.80-35.20 kg/ha) contents in Balasore soils but sandy loam soil enriched with higher TOC (0.45%) and AK (102.06 kg/ha) in NRRI soils would disfavour PSB population in former but support in latter fields. However, more AP (27-33 kg/ha) but lesser PSB in Balasore and lower AP (22.20 kg/ha) but more PSB in NRRI soils (Table 1) are unusual and unexplainable from this study. The PSB populations of NRRI and Balasore soils conformed to 7.05×10^5 cfu/g of other irrigated rice fields (Subhashin and Padmaja, 2011) but were significantly lower than (10-20) x 10^8 cfu/g PSB of lowland rice soils of Port Blair, Mahe and Mangalore coasts and Srinagar which indicated that organic matter decomposition or nutrient accumulation in lowland would support more microbes (including PSB) than the fields of upper topogradient.

The 6 potent PSB produced different types of colonies; the bacteria were Gram positive, spore forming, motile rods measuring 2.05-3.8 x 1.4-2.35 µm sizes (Table 3 & Fig. 2, 3 and 4). Besides, the organisms possessed catalase (except for AP 2), urease (except for AP 2 and AP 6), citrate utilization (AP 7 only) and esculin hydrolyzing properties but did not produce indole, AMC (except for AP 2), nitrate reductase (except for AP 2) and other test results were variable (Table 3).

Table 4. Genomic and plasmid profile of the bacteria

Isolate no.	Genomic DNA	Plasmid	
	Molecular weight (kDa)	No.	Size (kbp)
AP2	14	4	37.9, 30.8, 22, 6.3
AP7	14.1	ND	ND
AP8	12.8	ND	ND
AP9	14.3	3	37.9, 20.5, 8.8
AP12	13.2	4	37.9, 29.3, 22.2, 5.8

*ND= not done

Table 5. Cellular protein profile of the bacteria

Isolate no.	Protein No.	Size (kDa)
AP7	15	305.3, 241, 225, 123.1, 94.5, 90.6, 84.1, 64, 61, 58.6, 51.4, 42.8, 41.6, 33.9, 32.2
AP8	13	305.3, 257.1, 230.3, 131.8, 65.9, 61.6, 59.2, 53.5, 44.9, 42, 34.1, 36.6, 32.8
AP9	10	337.5, 246.4, 140.8, 68.5, 63.4, 54.6, 44.9,42.4, 34.2, 33.1

Table 6. Restriction digestion profile of 16S rDNA of the bacteria

Isolate no.	Size (kbp)		
	BamHI	EcoRI	HaeIII
AP7	Nil	0.91, 0.71	0.64, 0.47
AP8	Nil	0.91, 0.68	0.67, 0.50
AP9	Nil	0.90, 0.71	0.64, 0.47
AP12	Nil	0.91, 0.73	0.64, 0.48

AP 2 produced gas from all carbon compounds (except for arabinose), acid from fructose unlike from sucrose by other bacteria and the remainder organisms had differential responses (Table 3). The genomes of the organisms were 12.8-14 kDa and possessed 0-4 plasmids of various sizes (Table 4) which indicated their genetic diversity and proved that plasmid composition is not strain specific and would vary from cryptic to mega-plasmid sizes (Logan et al., 2009; Fagundes et al., 2011). Nevertheless the phenotypic and genetic results were in agreement with the characters of the Family Firmicutes (de Vos et al., 2009) and identified the organisms as *Bacillus cereus* (AP 2), *B. pantothenticus* (AP 6), *B. macquariensis* (AP 7), B. badius (AP 8 and AP 9) and *B. sphaericus* (AP 12) (Logan et al., 2009; Krieg, 2015). Growth of the organisms on NA with or without salt indicated that the organisms would be halotolerant (Zahran, 1997; Das and Dangar, 2008). As antibiotic sensitivity is not a universal property of any bacterial strains (Krieg, 1984), the PS *Bacillus* spp. would response differentially to the tested antibiotics (Table 3).

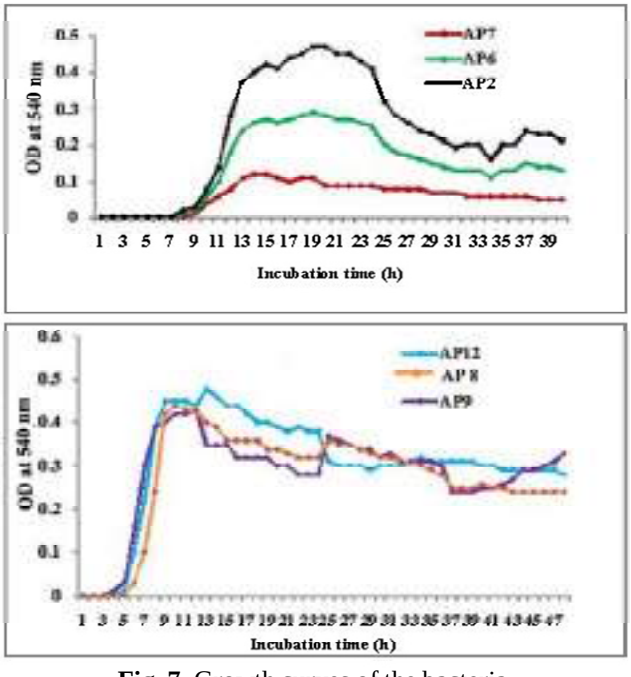

Fig. 7. Growth curves of the bacteria

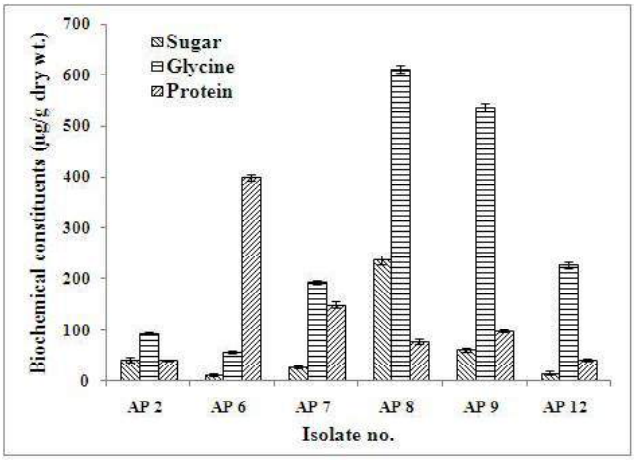

Fig. 8. Contents of organic constituents of the bacteria

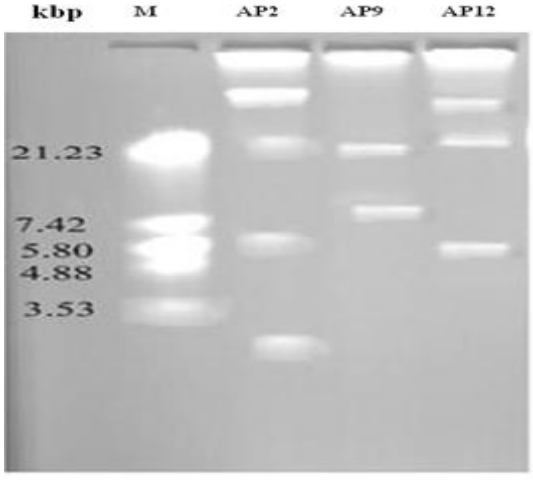

Fig. 9. Plasmid profile of the potent isolates

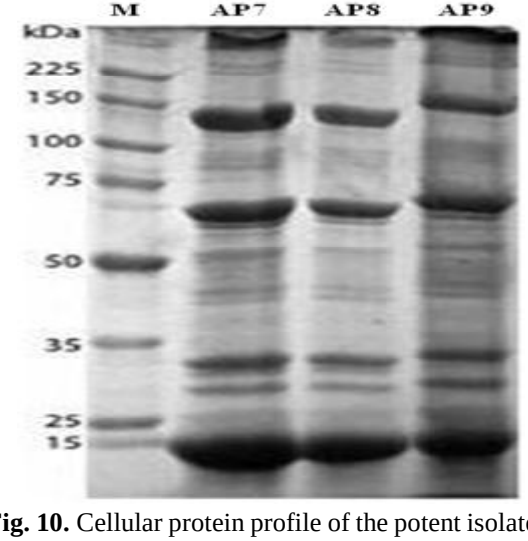

Fig. 10. Cellular protein profile of the potent isolates

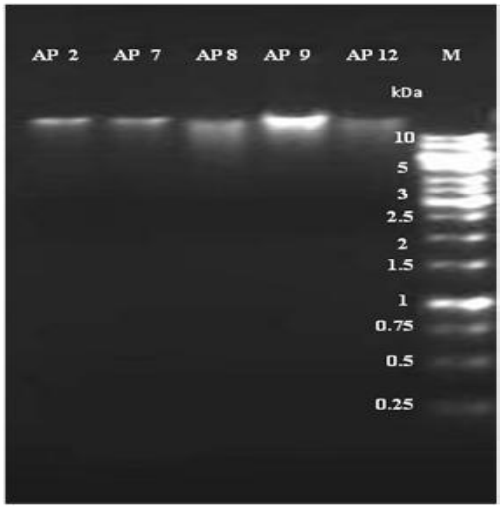

Fig. 11. Genomic DNA profile of the isolates

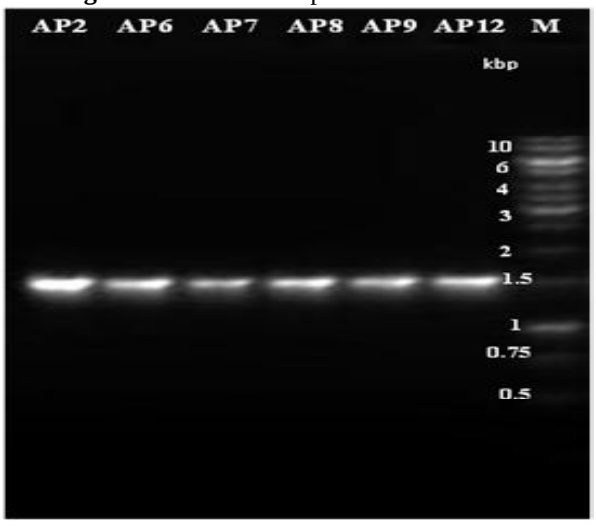

Fig. 12. 16S rDNA amplification

The PS bacilli produced 10-15 proteins of 32.2-305.3 kDa sizes (Table 5) depicting the inter species differences of protein composition of the PS bacilli and corroborated the reports of different other bacterial genera including *Bacillus* (Logan et al., 2009). The isozymes of amylases produced more than 10 bands but only one for catalase (Figs. 14 and 15) proved that the PSB did not have uniform metabolic make up. Diversity of amylase with several isozymes indicated that they would be potent decomposers of organic carbohydrate polymers and help in nutrition cycling to maintain soil nutritional qualities. Absence of recognition sites on 16S rDNA for *BamHI* but digestion by *EcoRI* and *HaeIII* into 2 comparable fragments (Table 6) proved identical recognition sites of 16S rDNA each of them and proved that V3 hypervariable region of 16S rRNA gene is most suitable for distinguishing all bacterial species to the genus level (Mizrahi-Man et al., 2013). Cellular constituents were highly variable among the organisms (Fig. 8). Sugar (237.5 µg/g dr. wt.) and glycine (612.5 µg/g dr. wt.) contents were more in AP 8 but AP 6 had minimum sugar (12.3 µg/g dr. wt.) and glycine (58.7 µg/g dr. wt.) and maximum protein (400 µg/g dr. wt.), but AP 2 had minimum protein (42.5 µg/g dr. wt.) (Fig. 8). The results proved that physiological difference is intrinsic character of bacteria and agreed to the observation in *B. thuringiensis* (Das and Dangar, 2008). Out of the two (*pqqAB12* and *pqqCF34*) P- solubilization genes, AP 2 contained both of them, whereas, AP 2, AP 6, AP 9 and AP 12 contained

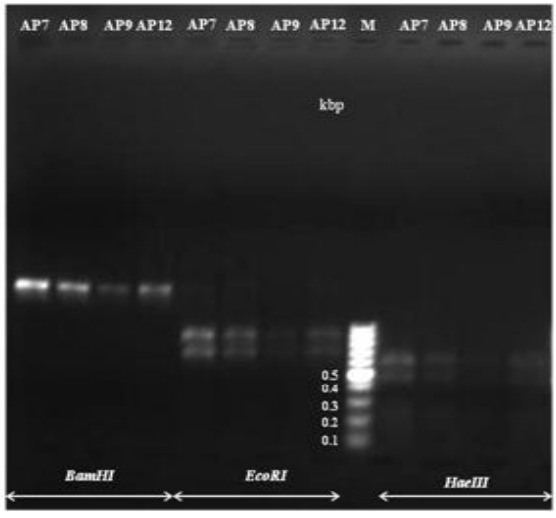

Fig. 13. Restriction digestion profile of 16S rDNA of the potent isolates

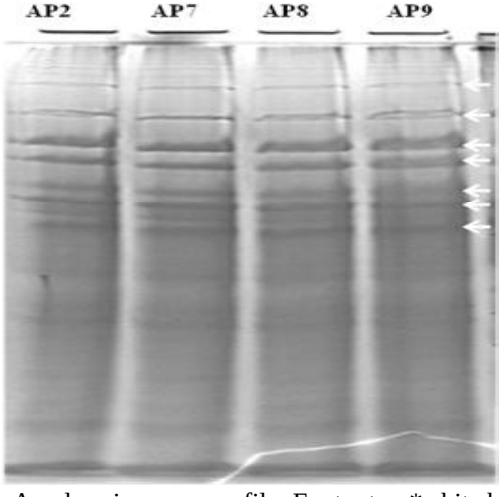

Fig. 14. Amylase isozyme profile. Footnote - *white bands represent amylase activity

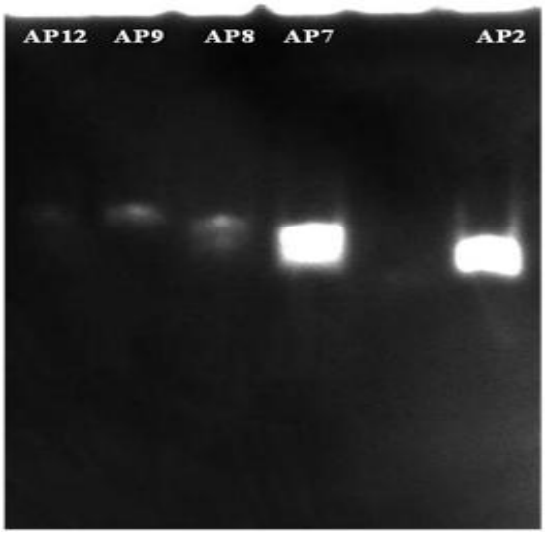

Fig. 15. Catalase isozyme profile

only *pqqCF34* gene (Fig. 16 and 17) which depicted that P solubilizing functions are also different in different PS bacilli.

All selected isolates (AP 2, AP 6, AP 7, AP 8, AP 9 and AP 12) solubilized phosphate, fixed nitrogen and produced ammonia (but AP 6) along with siderophore by AP 2, AP 9 and AP 12 (Table 2) which proved their polyvalent PGP functionality. The AP 8 solubilized maximum P (402 µg/g dry wt.) and effected maximum acidification (pH 4.17) after 3 d (Table 2; Fig. 5) indicating that it more efficiently follows acidification for P mineralization than others. Nevertheless, the PSB differed in PGP functions indicating wide diversity of the P-solubilizing *Bacillus*

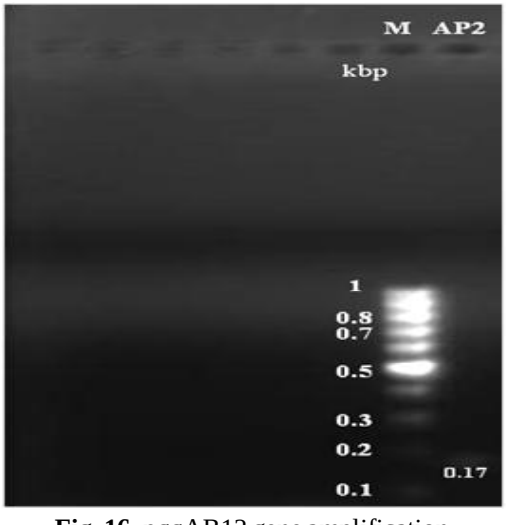

Fig. 16. pqqAB12 gene amplification

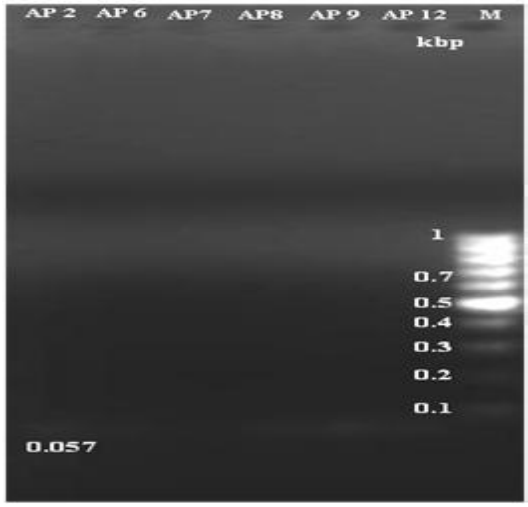

Fig. 17. pqqCF34 gene amplification

spp. in rice soils. However, alike the PS *Bacillus* spp., intra-species structural and functional differences are generally encountered, especially in the wild bacteria (Garrity, 2001; Tilak et al., 2005; Subhashin and Padmaja, 2011; Stephen et al., 2015). The results envisaged that PS *Bacillus* spp. are diverse in Indian coastal rice soils and some of the isolates would be potent for maintenance of soil nutrition and crop productivity.

ACKNOWLEDGEMENT

Financial support from AMAAS, ICAR, India is acknowledged.

CONCLUSION

The phosphate solubilizing bacterial population in the coastal saline soils was comparable to other mesophilic soils. The potent organisms belonged to the family Firmicutes and order Bacillaceae. The indigenous *Bacillus* spp. were diverse in physiological and genetic characters, osmotic stress tolerance, molecular compositions and antibiotic sensitivity. The organisms were halotolerant and therefore, they can be exploited for maintenance of soil health and nutrition in saline ecologies and control of plant pathogens.

REFERENCES

Bakker AW and Schippers B (1987). Microbial cyanide production in the rhizosphere in relation to potato yield reduction and *Pseudomonas* spp. mediated plant growth stimulation. Soil Biol. Biochem. 19(4): 451-457

Bric JM, Bostock RM and Silverstone SE (1991). Rapid in situ assay for indoleacetic-acid production by bacteria immobilized on a nitrocellulose membrane. Appl. Environ. Microbiol. 57 (2): 535-538

Collee J and Miles RS (1989). Tests for identification of bacteria. In: College JR, Duguid JP, Fraser AG, Marmion BP (eds). Mackie and McCartney's Practical Medical Microbiology. 13th ed. Churchill Livingstone/Longman, London pp. 141-160

Das J and Dangar TK (2008). Microbial population dynamics, especially stress tolerant *Bacillus thuringiensis*, in partially anaerobic rice field soils during postharvest period of the Himalayan, island, brackish water and coastal habitats of India. World J. Microbiol. Biotechnol. 24(8): 1403-1410

de Vos P, Garrity G, Jones D, Krieg NR, Ludwig W, Rainey FA, Schleifer KH and Whitman W (Eds.) (2009). Bergey's Manual of Systematic Bacteriology, Vol. 3: The Firmicutes. New York, Springer

Fagundes RBS, Picoli EAT, Lana UGP and Valicente FH (2011). Plasmid patterns of efficient and inefficient strains of *Bacillus thuringiensis* against Spodoptera frugiperda (J.E. Smith) (Lepidoptera: Noctuidae). Neotrop. Entomol. 40(5): 600-606

Garrity GM, Winters M and Searles DB (2001). Taxonomic Outline of the Prokaryotic Genera. Bergey's Manual of Systematic Bacteriology, 2nd Edition. Release 1.0: 1-39, Springer, New York

Jensen GB, Wilcks A, Petersen S, Damgaard J, Baum JA and Andrup L (1995). The genetic basis of the aggregation system in *Bacillus thuringiensis* subsp. *israelensis* is located on the large conjugative plasmid pX016. J. Bacteriol. 177(10): 2914-2917

Krieg NK (1984). Bergey's Manual of Systematic Bacteriology, Vol. 1. Williams and Wilkins, Baltimore, Hong Kong, London, Sydney

Krieg NR (2015). Bacteroidia class. nov.. Bergey's Manual of Systematics of Archaea and Bacteria. Springer, New York

Lacey LA (1997). Manual of techniques in insect pathology. Academic Press, New York

Logan NA, de Vos P and Genus I (2009). In: De Vos P, Garrity GM, Jones D, Krieg NR, Ludwig W, Rainey FA, Schleifer KH, Whitman WB (Eds.), *Bacillus* Cohn 1872, 174AL, 2nd edition. Bergey's Manual of Systematic Bacteriology, 3. Springer, New York, pp. 21-128

Mizrahi-Man O, Davenport ER and Gilad Y (2013). Taxonomic Classification of Bacterial 16S rRNA Genes Using Short Sequencing Reads: Evaluation of Effective Study Designs. PLoS ONE, 8(1): e53608. doi:10.1371/journal.pone.0053608

Olsen SR, Cole CV, Watanabe FS and Dean LA (1954). Estimation of available phosphorus in soil by extraction with sodium bicarbonate. USDA Circ. 939. U.S. Government Printing Office, Washington, D.C.

Ponmurugan P and Gopi C (2006). Distribution pattern and screening of phosphate solubilising bacteria isolated from different food and forage crops. J. Agron. 5(4): 600-604

Premono ME, Moawad AM and Vlek PLG (1996). Effect of phosphate-solubilizing Pseudomonas putida on the growth of maize and its survival in the rhizosphere. Indones. J. Agric. Sci. 11: 13-23

Reed RH, Richardson DL, Warr SRC and Stewart WDP (1984). Carbohydrate accumulation and osmotic stress in cyanobacteria. J. Gen. Microbiol. 130: 1-4

Saber K, Nahla LD and Chedly A (2005). Effect of P on nodule formation and N fixation in bean. Agron. Sustain. Dev. 25: 389-393

Schwyn B and Neilands JB (1987). Universal chemical assay for the detection and determination of siderophore. Anal. Biochem. 160(1): 47-56

Smibert RM and Krieg NR (1995). Phenotypic characterization. In: Gerhardt P, Murray RGE, Wood W, Krieg E (eds) Methods for general and molecular bacteriology. American Society for Microbiology, Washington pp. 607-654

Smyth EM, McCarthy J, Nevin R, Khan MR, Dow JM, O'Gara F and Doohan FM (2011). In vitro analyses are not reliable predictors of the plant growth promotion capability of bacteria; a *Pseudomonas fluorescens* strain that promotes the growth and yield of wheat. J. Appl. Microbiol. 111(3): 683-692 doi:10.1111/j.1365-2672. 2011.05079.x

Stephen J, Shabanamol S, Rishad KS and Jisha MS (2015). Growth enhancement of rice (*Oryza sativa*) by phosphate solubilizing *Gluconacetobacter* sp. (MTCC 8368) and *Burkholderia* sp. (MTCC 8369) under greenhouse conditions. 3 Biotech. 5(5): 831-837

Subhashin DV and Padmaja K (2011). Population dynamics and screening of phosphate-solubilizing bacteria isolated from tobacco (*Nicotiana tabacum*)-based cropping systems. Indian J. Agr. Sci. 81(8): 740-743

Thimmaiah SR (1999). Standard methods of biochemical analysis. pp. 230-231. Kalyani Publishers, New Delhi

Tilak KVBR, Ranganayaki N, Pal KK, De R, Saxena AK, Nautiyal CS, Mittal S, Tripathi AK and Johri BN (2005). Diversity of plant growth and soil health supporting bacteria. Curr. Sci. 89(1): 136-150

Vassilev N and Vassileva M (2003). Biotechnological solubilization of rock phosphate on media containing agro-industrial wastes. Appl. Microbiol. Biotechnol. 61(5): 435-440

Zahran HH (1997). Diversity adaptation and activity of the bacterial flora in saline environments. Biol. Fertil. Soils. 25(3): 211-223

Oryza Vol. 54 No. 2, 2017 (220-226)
DOI 10.5958/2249-5266.2017.00028.5

Market outlook of major paddy markets in India

M Srikala[1*], I Bhavani Devi[2], S Rajeswari[1], G Mohan Naidu[1] and SV Prasad[1]

[1]S.V.Agricultural College, Tirupati -517 502, Andhra Pradesh, India

[2]Institute of Agribusiness Management, ANGRAU, Tirupati-517502, Andhra Pradesh, India

**Corresponding author e-mail: srikalamedikondu@gmail.com*

Received : 26 May 2016 Accepted : 04 August 2017 Published : 28 September 2017

ABSTRACT

Monthly modal prices of paddy corresponding to Nizamabad and Suryapet markets became stationary at level as well as at first difference, where as the prices of Sindhanur and Toofanganj markets became stationary only after first differencing. Johansen's multiple co-integration tests revealed long-run equilibrium relationship among the markets. The causality test revealed a bi-directional influence of paddy prices between Sindhanur and Nizamabadand, Suryapet and Nizamabadand and Sindhanur and Suryapet markets. The Toofanganj market prices have depicted uni-directional causality on the prices of Nizamabad and Sindhanur and Suryapet market prices have shown uni-directional causality with Toofanganj market prices. Nizamabad market came to short run equilibrium within 24 days as indicated by co-efficient values. The results of Vector Error Correction Model (VECM) showed that most of the markets considered under this study were integrated to each other.

Key words: *Augmented dickey fuller, granger causality, long-run equilibrium, market integration, vector error correction model*

INTRODUCTION

Paddy is the most important and extensively grown cereal crop in the world. It is the staple food of more than 60 per cent of the world's population. One-fifth of the world's population *i.e.*, more than a billion people depend on rice cultivation for their livelihoods. Rice is produced in about 42 countries and recognized as staple food in Asia, Latin America, parts of Africa, Middle East and serve as a primary source of food for more than half of the world's population. The world rice market is also featured by a high degree of concentration as the data shows that most of the rice produced is consumed domestically, only 7 per cent of global production is internationally traded (FAO, 2012). Rice is not a uniform commodity and consumer preferences for specific types and qualities are often well-established so there is limited scope for substitution in rice (FAO, 2004). Asia, where about 90 per cent of rice is grown, has more than 200 million rice farms, most of which are smaller than one hectare (FAO, 2012). Rice-based farming is the main economic activity for hundreds of millions of rural poor in this region.

The global area of rice was 157.46 million ha, with a production and productivity of 701.52 million tonne and 4.46 t/ha respectively in 2015 (www.usda.gov). China was the leading rice producer followed by India, Indonesia and Bangladesh in 2014-15 (www.agricoop.nic.in). India tops among the paddy growing countries globally for 2014-15 with an area of 42.75 million ha, production of 154.52 million tonnes and productivity of 3.61 tonne/ha (http://ricestat.irri.org:8080/wrs2/entrypoint.htm).

Agricultural commodities are typically produced over extensive spatial area and are costly to transport relative to their total value. Fackler and Goodwin (2002) noted that these characteristics of agricultural products yield a complex set of spatial price linkages which needed to be studied to gain insights into the performance of markets. The accuracy and speed at which price change in one market gets transmitted to other markets is taken as an indicator of integration

(inter dependence) among the markets. The extent of integration gives signals for efficient resource allocation, which is considered essential for ensuring greater market efficiency, price stability and food security. Test of integration also plays a key role in determining the geographical level at which agriculture price policy should be targeted, at least in the short-run to ensure regular availability of food and price stability (Jha et al., 2005). If price changes in one market are fully reflected in an alternate market then these markets are said to be spatially integrated, it indicates the overall market performance. In an integrated market, prices of a commodity are responsive to price changes of the same quality products in other markets. As such price differences of a particular variety of product in different markets of the area as a rule should not exceed the cost involved in transportation and handling of the produce. The ultimate objective of the planners and policy makers in the field of agricultural marketing is to develop efficient markets for the agricultural produce produced by the farmers. The market integration concept explains the relationship between the prices in two markets that are spatially separated. When markets are integrated it implies that the markets in the system operate in uniform, as a single market system. The present study aims at studying the integration of paddy markets in India.

MATERIALS AND METHODS

The volume of transactions of paddy (Sona masuri) was the basis for selecting major markets. This criterion led for the selection of four major markets, Nizamabad and Suryapet (Andhra Pradesh), Sindhanur (Karnataka) and Toofanganj (West Bengal) were selected for the study purposively.

Before analysing any time series data, testing for stationarity is necessary (Davidson and Mackinnon, 1993) since the data has the presence of trend components. Time series stationarity is the statistical characteristics of a series such as its mean and variance over time. If both are constant over time, then the series is said to be stationary *i.e.*, there is no random walk or unit root. If the series was found to be non-stationary, then the first differences of the series were to be tested for stationarity. The number of times (d) a series was differenced to make it stationary is referred as the order of integration, I (d). The Augmented Dickey Fuller (ADF) test was applied by running the regression of the following formula

$$\Delta Y_{t\,i} = B\beta_1 + \delta Y_{ti\,-\,1} + \alpha_i \sum \Delta Y_{t\,-\,1} + e_t \qquad ...(1)$$

where, Y_i denoted price series of paddy markets and *i*=1,2,...5 (1- Nizamabad, 2-Suryapet, 3-Sindhanur and 4-Toofanganj). For a series to be stationary β must be less than unity in absolute value. Hence stationarity requires that $-1 < \beta < 1$ (Vavra & Goodwin, 2005). A series without differencing is integrated of order zero; denoted as I (0) while a series stationary at first difference is integrated of order one, denoted as I (1). Once the variables were checked for stationarity and were of same order, integration between them could be tested using Johansen maximum likelihood test.

Two series are said to be co-integrated when there exists a long run equilibrium relationship between them. In other words, two series cannot drift from one another in the long run. That is, there exists an equilibrium mechanism to bring the two series together. Applying this concept to any two given markets, co-integration between their price series implies long run dependence between them. Since the very essence of market integration is the price dependence across markets, it follows that co-integration between prices in two given markets implies integration of the markets.

To examine the price relation between two markets, the following basic relationship commonly used to test for the existence of market integration may be considered.

$$P_{it} = \alpha_0 + \alpha_1 P_{jt} + \varepsilon_t \qquad ....(2)$$

Where,

P_i = Price series of paddy in ith market.

P_j =Price series of paddy in jth market.

ε_t = is the residual term assumed to be distributed identically and independently

α_0 = represent domestic transportation costs, processing costs and sales taxes.

The test of market integration is straight forward

if P_i and P_j are stationary variables.

Often, however, economic variables are non - stationary in which case the conventional tests are biased towards rejecting the null hypothesis. Thus, before proceeding to further analysis, it is important to check for the stationarity of the variables.

If price series are I (1), then one could run regressions in their first differences. However, by taking first differences, the long-run relationship that is stored in the data is being lost. This implies that one needs to use variables in levels as well. Advantage of the Error Correction Model (ECM) is that it incorporates variables both in their levels and first differences. By doing this, ECM captures the short-run dis-equilibrium situations as well as the long-run equilibrium adjustments between prices. ECM can incorporate such short-run and long-run changes in the price movements. A generalized ECM formulation to understand both the short-run and long-run behaviour of prices can be considered by first taking the Autoregressive Distributed Lag (ADL) equation as follows:

$$Y_t = \alpha_{01}\ X_t + a_{11}X_{t-1} + a_{12}Y_{t-1} + \varepsilon_t$$

$$\Delta Y_t = a_{01}\Delta X_t + (1 - a_{12})\left[\frac{(a_{01} + a_{11})}{(1 - a_{12})}\ X_{t-1} - Y_{t-1}\right] + \varepsilon_t$$

The generalized form of this equation for k lags and an intercept term is as follows:

$$\Delta Y_t = a_{00} + \sum_{i=0}^{k-1} a_{i1}\Delta X_{t-i} + \sum_{i-1}^{k-1} a_{i2}\Delta Y_{t-i} + m_0[m_1 X_{t-k} - Y_{t-k}] + \varepsilon_t$$

where $$m_0 = (1 - \sum_{i=1}^{k} a_{i2}), and\ m_1 = \frac{\sum_{i=0}^{k} a_{i1}}{m_0}$$

The parameters m_0 measures the rate of adjustment of the short-run deviations towards the long run equilibrium. Theoretically, this parameter lies between 0 and 1. The value 0 denotes no adjustment and 1 indicates an instantaneous adjustment. A value between 0 and 1 indicates that any deviations will have gradual adjustment to the long-run equilibrium values.

Granger Causality Test

Granger (1969) developed a methodology to examine whether changes in one series cause changes in another. If the current value of Y can be predicted by using the past values of X and considering other relevant information including the past values of Y, it may be concluded that X causes Y. Similarly, if the current values of X can be predicted by considering past values of Y and past values of X, it is concluded that Y causes X. The following two OLS regressions used in Granger causality test explains the above concept.

$$Y_i = a_i Y_{t-i} + b_i X_{t-i} + e_i \ldots\ldots\ldots 3$$

$$X_i = c_i Y_{t-i} + d_i X_{t-i} + v_i \ldots\ldots\ldots 4$$

Where, X_i and Y_i are two stationary time series with zero mean: e_i and v_i are two correlated series. Since the series of the variable are usually non-stationary and integrated of order I (1), first difference of the variable is normally taken which is stationary. The optimal lag length of the variables is determined by minimising Akaike's Information Criterion (AIC). Based on equations 3 and 4, uni-directional causation from one variable X to Y (*i.e.,* X Granger causes Y) is observed if the estimated coefficient on the lagged X variable in equation 3 is statistically non-zero as a group and the set of lagged Y coefficient is zero in equation 4. Similarly, uni-directional causation from Y to X (*i.e.,* Y Granger causes X) is implied if the estimated coefficient on the lagged Y in equation 4 are statistically different from zero as a group and the set of estimated coefficient on the lagged X variable in equation 3 is not statistically different from zero. Feedback or mutual causality (bi-directional) would occur when the set of coefficients on the lagged X variable in equation 3 and on lagged Y variable in equation 4 are statistically different from zero. Finally, independence exists when the coefficients of both X and Y variables are equal to zero.

For the present study, to analyse the integration among the selected markets monthly modal price data for the period from January 2005 to December 2015 for paddy crop was utilised.

RESULTS AND DISCUSSION

Augmented Dickey Fuller (ADF) Test

Before conducting co-integration tests, it is needed to examine the uni- variate time-series properties of the data and confirm that all the price series are stationary and integrated with the same order. This is performed