RESEARCH PAPER | Asian Journal of Bio Science, Volume **8** | Issue 1 | April, 2013 | 63-68
Received : 26.12.2012; **Revised :** 15.02.2013; **Accepted :** 17.03.2013

Screening of diazotrophic bacterial communities from wild rice (*Oryza indica*) and cultivated rice (*Oryza sativa*) and their plant growth promoting activities

C. SARATHAMBAL[1], K. ILAMURUGU[1], S. ARUN[2] **AND** L. SRIMATHI PRIYA[1]

[1]Department of Agricultural Microbiology, Tamil Nadu Agricultural University, COIMBATORE (T.N). INDIA
[2]Department of Soil Science and Agricultural Chemistry, Tamil Nadu Agricultural University, COIMBATORE (T.N.) INDIA

Email: saratha6@gmail.com; ilamuruguk@gmail.com; arun99005@gmail.com; agrisriya@gmail.com

A study was undertaken to screen the free living, culturable diazotrophic bacteria from wild rice (*Oryza indica*) and cultivated rice (*Oryza sativa*) and their plant growth promoting activities. Out of forty eight diazotrophic isolates, thirty eight isolates recorded positive growth in N-free medium which were further analyzed for total nitrogen and ammonia. Based on total nitrogen and ammonia production, twenty eight diazotrophic isolates were selected for nitrogenase activity. The highest nitrogenase activity was exhibited by isolate GDR16 (4134±56.6 nm of ethylene mg^{-1} $protein^{-1}$ hr^{-1}). For PGPR activity 11 isolates from *O.indica* and 9 isolates from *O.sativa* with elite nitrogenase activity were selected for PGPR as well as mineral solubilization studies. Out of these 20 diazotrophic isolates, 11 isolates showed IAA production. The maximum amount of IAA was produced by CBE1 (35.5 ±1.14µg ml^{-1}). The highest amount of GA was produced by GDR13 (21.7±0.19 µg ml^{-1}), followed by GDR 7 which produced 18.4±0.23 µg ml^{-1}. The maximum siderophore production was recorded with CBE1 (43.94 ±0.64µg mg^{-1} dry weight of cell of catechol type). With respect to mineral solubilization, 17 were able to solubilze the insoluble phosphorus and 7 were able to solubilze the zinc. The results of the present study showed the diazotrophic bacteria associated to both wild and cultivated rice and it having variety of plant growth promoting substances in considerable amounts apart from diazotrophy.

Key words : Rice, Diazotrophs, *Oryza indica*, *Oryza sativa*, PGPR

How to cite this paper : Sarathambal, C., Ilamurugu, K., Arun, S. and Priya, L. Srimathi (2013). Screening of diazotrophic bacterial communities from wild rice (*Oryza indica*) and cultivated rice (*Oryza sativa*) and their plant growth promoting activities. *Asian J. Bio. Sci.*, **8** (1) : 63-68.

INTRODUCTION

Rice (*Oryza sativa* L.) is the staple diet of over 40 per cent of the world's population making it the most important food crop currently produced (Hossain and Fischer, 1995). Much of this rice is grown in countries where rapidly growing populations, coupled to limited amounts of land and scarce resources, make high yields per hectare with reduced inputs essential to avoid food shortages. Rice shows a remarkable diversity because of its long history of cultivation and because of its selection under various climatic, edaphic and biotic environments in geographically diverse areas (Lu and Chang, 1980). It is arguably the most important crop among the world's inhabitants, that enhances nitrogen fixation during the rice root-bacterial association under flooded soil conditions and attracted a great deal of interest for a long time. Crop productivity is based on numerous variables including weather, soil type, moisture and nutrients. One of the most important factors in the generation of high yields from modern rice crops is the nitrogen fertilizer, without which yield of the present varieties is drastically limited. Inspite of biological nitrogen fixation in wetland rice fields contributes significantly to the long term fertility of these systems (Roger and Ladha, 1992).

Based on these studies, several species of diazotrophs, such as *Klebsiella* (Ladha *et al*., 1983; Fujii *et al*., 1987), *Alcaligenes* (You and Zhou, 1989) and *Azospirillum* (Baldani and Dobereiner, 1980) have been isolated from the rhizosphere of wetland rice. In contrast, wild species of *Oryza indica* (wild rice) grow in marshlands of the tropics and subtropics where most of these survive as perennial plants (Sato, 1994). Therefore, wild rice are likely to harbour unique populations of bacteria that differ from those in extensively bred modern varieties of rice subjected to the application of various

fertilizers and agrochemicals. Hence, the present study was focused on the isolation and screening of diazotrophic bacteria from wild and cultivated rice variety and their plant growth promoting activities.

RESEARCH METHODOLOGY

Soil sampling and isolation of diazotrophs:

Rhizosphere soils of wild rice (*Oryza indica*) and cultivated rice variety ADT 45 (*Oryza sativa*) were collected from the Gudulur, Ooty (Western Ghats) and wet land, Tamil Nadu Agricultural University (Coimbatore). Plants were uprooted carefully and the soil adhering to the roots was collected in a sterile Petri dish and mixed thoroughly so as to make a composite sample for microbiological analysis. The samples were taken to physio-chemical analysis and stored at 4^0C for further studies (Pramer and Schmidt, 1966). Diazotrophs were isolated using serial dilution technique on three selective N-free media *viz.,* NFMM (Piao *et al*., 2005), LGI-P (Reis *et al.,* 1994) and JNFb (Kirchhof *et al.,* 1997). Morphologically different, single, well-separated colonies on the plates were picked, purified on solid N free medium, transferred into agar slants and preserved at 4^0C for preliminary screening.

Screening for diazotrophy of the isolates:

The diazotrophs isolated from different rice rhizospheres were tested for their ability to grow in N-free medium (Burris and Wilson,1972). Those isolates which had positive value in N- free medium were further analyzed for total nitrogen (Humphries,1956) and ammonia production (Cappuccino and Sherman, 1992).

Nitrogen fixation of the diazotrophs were determined by acetylene reduction assay (ARA) using gas chromatograph (Chemito-7610) fitted with flame ionization detector and a Porapak-N column (Park *et al.,* 2005). All the experiments were carried out in semisolid JNFb medium. Uninoculated media served as control. The protein concentration was determined using bovine serum albumin as standard (Lowry *et al*., 1951).

Screening of diazotrophs for multifaceted-plant growth promoting activities:

An aliquot was taken from each pure culture for evaluation of PGPB characteristic (indol 3-acetic acid, gibberellic acid identification and quantification, siderophore production and mineral solubilization.

Indole Acetic Acid (IAA) estimation and gibberellic acid (GA_3) production was done using the method of Chandramohan and Mahadevan (1968) and Borrow *et al.* (1955), respectively. Siderophore production was checked using the Chrome Azurol S (CAS) agar plates (Dubey and Maheshwari, 2004). Solubilization of insoluble phosphates (Katznelson and Bose, 1959) and zinc (Bunt and Rovira, 1955) were also assayed.

RESEARCH FINDINGS AND ANALYSIS

In our attempt to screen the diazotrophic bacteria, 2 species of wild and cultivated rice variety were used. Various physio-chemical properties of rice rhizosphere soils *viz.,* pH, EC, texture, available nitrogen, phosphorus and potassium from two different locations were estimated and are presented in Table 1. Out of forty eight diazotrophic isolates, thirty eight isolates recorded positive growth in N- free medium which were further analyzed for total nitrogen and ammonia (Table 2). Total N determination using microKjeldhal distillation apparatus exhibited variation from 1.2 ± 0.01 to 14.50 ± 0.12 mg N g^{-1} malate, as mentioned in Table 2. The diazotrophic isolate GDR6 was found to produce the maximum total nitrogen content (14.50 ± 0.89 mg N g^{-1} malate). Sgroy *et al.* (2009) demonstrated the nitrogen fixing ability of *Bacillus subtilis*, *B. pumilis*, *Brevibacterium halotolerans* and *Pseudomonas putida* by growing the isolates in nitrogen free medium, as qualitative evidence of atmospheric nitrogen fixation. All isolates were able to grow in nitrogen-free culture medium, and this capability could be attributed to the acquisition of atmospheric nitrogen by biological fixation. Nitrogen fixation capacity of diazotrophs was assessed indirectly by measuring the products of nitrogen fixation activity *viz.,* extracellular protein and ammonia concentrations.

The diazotrophic isolates were tested for their ability to produce ammonia both qualitatively and quantitatively. The results of ammonia production by the diazotrophic isolates are presented in Table 2. The quantity of ammonia production varied between 1.1 ± 0.04 to 4.6 ± 0.12 mg ml^{-1} where, the isolate GDR14 produced maximum of 4.6 ± 0.26 mg ml^{-1}. Mortenson (1951) devised a rapid method for measuring nitrogen fixation by measurement of ammonia produced during nitrogen fixation by cell-free enzyme preparations. Concern with the quantitative analysis for the intermediates in biological N_2 fixation is centered on ammonia because it is the only demonstrated intermediate in the process (Burris, 1972).

Based on total nitrogen and ammonia production, twenty

Table 1 : Physio-chemical properties of rhizosphere soils of *Oryza indica* and *Oryza sativa* (ADT -45)

Soil sample	Soil pH	EC (dSm^{-1})	Organic carbon (g kg^{-1})	Available nutrients (g kg^{-1})		
				N	P	K
O. indica	8.02	0.42	3.26	256	26.9	536
O. sativa	8.14	0.26	2.56	235	19.8	449

Table 2: Growth on N-free medium, total nitrogen content and ammonia production of the rhizosphere diazotrophic isolates

Sr. No.	Isolates	Growth @ 660nm	Total nitrogen mg/g of malate	Ammonia (mg ml^{-1})
1.	GDR1	++	10.3(±0.12)^{cd}	3.2 (±0.24)^{b}
2.	GDR2	+	5.3 (±0.13)^{fg}	1.3 (±0.10)^{klm}
3.	GDR3	+	ND	ND
4.	GDR4	++	6.3 (±0.14)^{f}	2.8 (±0.17)^{bcd}
5.	GDR5	+++	9.4 (±0.01)^{d}	2.7 (±0.16)$^{b-e}$
6.	GDR6	++	14.5 (± 0.12)^{a}	2.5 (±0.14)^{def}
7.	GDR7	++	9.2 (±0.35)^{de}	2.6 (±0.23)$^{c-f}$
8.	GDR8	++	10.4(±0.16)^{cd}	2.4 (±0.14)$^{d-g}$
9.	GDR9	++	2.1 (±0.48)^{hi}	1.9 (±0.20)$^{g-j}$
10.	GDR10	+++	2.8 (±0.42)^{hi}	1.2 (±0.10)^{lm}
11.	GDR11	+	ND	ND
12.	GDR12	+	ND	1.1 (±0.04) ^{m}
13.	GDR13	+	6.8 (±0.53)^{f}	1.3 (±0.10)^{klm}
14.	GDR14	+	9.6 (±0.10)^{d}	4.6 (±0.26)^{a}
15.	GDR15	+	5.8 (±0.22)^{f}	2.5 (±0.24)^{def}
16.	GDR16	+	1.2 (± 0.01)^{i}	ND
17.	GDR17	+	ND	ND
18.	GDR18	++	1.6(±0.56)^{hi}	ND
19.	GDR19	+	ND	ND
20.	CBE1	+	7.2 (±0.58)^{ef}	ND
21.	CBE2	++	10.3 (±0.69)^{cd}	1.5 (±0.08)$^{j-m}$
22.	CBE3	+++	13.4 (±0.89)^{ab}	2.4 (±0.13)$^{d-g}$
23.	CBE4	+++	10.5(±0.98)^{cd}	1.9 (±0.13)$^{g-k}$
24.	CBE5	+	3.6 (±0.78)^{gh}	1.3 (±0.09)^{klm}
25.	CBE6	+++	11.4 (±0.42)^{bcd}	2.7 (±0.23)$^{b-e}$
26.	CBE7	+	ND	ND
27.	CBE8	+	2.2 (±0.20)^{hi}	ND
28.	CBE9	+++	7.2 (±0.24)^{ef}	1.6 (±0.12)$^{i-m}$
29.	CBE10	+	7.3 (±0.84)^{ef}	3.1 (±0.12)^{bc}
30.	CBE11	++	6.6 (±0.70)^{f}	2.2 (±0.15)$^{e-h}$
31.	CBE12	+	11.5 (±0.14)^{bcd}	ND
32.	CBE13	+	1.2 (±0.36)^{i}	3.1 (±0.19)^{bc}
33.	CBE14	+++	13.6 (±0.58)^{a}	2.5 (±0.28)^{def}
34.	CBE 15	+	12.5 (± 0.23)^{abc}	2.1 (±0.14)$^{f-i}$
35.	CBE16	++	6.6 (±0.78)^{f}	1.7 (±0.14)$^{h-l}$
36.	CBE17	+	ND	ND
37.	AZ 204*	++	5.50 (±0.60)^{fg}	1.7 (±0.13)$^{h-l}$

**Azospirillum lipoferum*, ND - not detected

Values are mean (± standard error) (n=3) and values followed by the same letter in each column are not significantly different from each other as determined by DMRT (p á 0.05).

eight diazotrophic isolates were selected for further investigation of nitrogenase activity. The highest nitrogenase activity was exhibited by isolate GDR16 (4134±56.6 nm of ethylene mg^{-1} $protein^{-1}$ hr) followed by GDR10 (3638±27.3 nm of ethylene/mg protein/hr). The standard culture *Azospirillum lipoferum* (strain AZ 204) showed nitrogenase activity of 896 ± 22.1 n moles of ethylene mg^{-1} of cells h^{-1} (Table 3).

In the recent decades there has been increasing evidence that besides N_2 fixation, synthesis and export of phytohormones by the N_2 fixing bacteria play an important

Table 3: Nitrogenase enzyme activity of diazotrophs obtained from rhizosphere soils of *Oryza indica* and *Oryza sativa* (ADT -45)

Sr. No.	Isolates	Nitrogenase activity nm of ethylene/mg protein/hr
1.	GDR1	2141(±86.6)^{de}
2.	GDR2	2577(±38.6)^{d}
3.	GDR5	ND
4.	GDR6	3163(±46.7)^{c}
5.	GDR7	1811(±96.4)^{ef}
6.	GDR8	1128(±26.8)^{ghi}
7.	GDR9	2302(±56.6)^{de}
8.	GDR10	3638(±27.3)^{b}
9.	GDR13	456(±16.9)^{jkl}
10.	GDR14	2536(±16.6)^{d}
11.	GDR15	ND
12.	GDR16	4134(±56.6)^{a}
13.	GDR18	325(±42.9)^{kl}
14.	GDR19	ND
15.	CBE1	ND
16.	CBE2	126(±33.9)^{l}
17.	CBE3	1589(±95.6)^{fg}
18.	CBE 4	654(±56.6)^{ijk}
19.	CBE5	189(±81.4)^{kl}
20.	CBE6	ND
21.	CBE8	ND
22.	CBE9	ND
23.	CBE10	456(±86.4)^{jkl}
24.	CBE11	923(±69.4)^{hij}
25.	CBE12	1634(±96.9)^{fg}
26.	CBE14	2564(±66.5)^{d}
27.	CBE 15	ND
28.	CBE16	1246(±25.2)^{gh}
29.	AZ 204*	896(±22.1)^{hij}

**Azospirillum lipoferum*, ND-not detected

Values are mean (± standard error) (n=3) and values followed by the same letter in each column are not significantly different from each other as determined by DMRT (p á 0.05).

role in the observed plant growth promotion. In this view, 11 isolates from *Oryza indica* and 9 isolates from *Oryza sativa* with elite nitrogenase activity were selected for PGPR as well as mineral solubilization studies. Out of these 20 diazotrophic isolates, 11 isolates showed IAA production. The maximum amount of IAA was produced by CBE1 (35.5 ±1.14µg ml^{-1}) followed by GDR13 (17.5 ±0.52 µg ml^{-1}) (Table 4). Beneduzi *et al*. (2008) reported that IAA production by *Bacillus* and *Paenibacillu*s from rice rhizosphere ranged from 0.1 and 30 ìg ml^{-1}. The highest amount of GA was produced by GDR13 (21.7±0.19 µg ml^{-1}), followed by GDR7 which produced 18.4±0.23 µg ml^{-1}. Of the 20 diazotrophic isolates, 14 isolates were able to produce siderophore. The maximum siderophore production was recorded with CBE1 (43.94 ±0.64µg mg^{-1} dry weight of cell of catechol type). The production of siderophore by many rhizosphere bacteria has been demonstrated to play an important role in biological control (Bagnasco *et al*., 1998). With respect to mineral solubilization, 17 were able to solubilze the insoluble phosphorus and 7 were able to solubilze the zinc. The results of the present study also revealed that most

Table 4: IAA and GA production, and mineral solubilsation by diazotrophic isolates

Sr. No.	Isolates	IAA (µg ml^{-1} of sample)	GA (µg ml^{-1} of sample)	Siderophore production (µg ml^{-1} of sample)	P	Zn
1.	GDR1	ND	ND	13.47(±0.18)^{f}	+	+
2.	GDR2	12.1(±0.44)^{cd}	9.8(±0.19)^{cde}	22.35(±0.10^{cde}	+	+
3.	GDR6	ND	ND	ND	-	-
4.	GDR7	13.3(±0.25)^{c}	18.4(±0.23)^{b}	ND	+	-
5.	GDR8	ND	7.6(±0.09)^{ef}	12.44(±0.24)^{fg}	+	+
6.	GDR9	9.7(±0.14)^{cde}	ND	38.43(±0.19)^{b}	+	-
7.	GDR10	7.4(±0.24)^{e}	5.3(±0.16)^{f}	24.50(±0.34)^{c}	+	+
8.	GDR13	17.5(±0.52)^{b}	21.7(±0.19)^{a}	13.4(±0.14)^{f}	+	-
9.	GDR14	13.2(±0.26)^{c}	ND	23.47(±0.11)^{c}	+	-
10.	GDR16	ND	8.1(±0.13)^{ef}	18.37(±0.70)^{e}	+	+
11.	GDR18	18.4(±0.59)^{b}	12.4(±0.12)^{cd}	10.41(±0.24)^{fg}	+	-
12.	CBE1	35.5(±1.14)^{a}	ND	43.94(±0.64)^{a}	+	-
13.	CBE2	ND	ND	8.41(±0.03)^{g}	+	+
14.	CBE3	ND	ND	11.43(±0.12)^{fg}	+	+
15.	CBE 4	12.3(±0.24)^{cd}	16.8(±0.10)^{b}	18.89(±0.13)^{de}	+	+
16.	CBE5	ND	ND	ND	-	-
17.	CBE10	8.2(±0.13)^{de}	9.6(±0.09)^{de}	ND	+	-
18.	CBE11	6.3(±0.10^{e}	8.9(±0.10)^{e}	ND	+	-
19.	CBE14	ND	ND	9.43(±0.11)^{fg}	+	-
20.	CBE16	ND	ND	ND	-	-
21.	Pf1*	8.3(±0.10)^{de}	12.9(±0.10)^{c}	13.47(±0.13)^{f}	+	-

*Pf1- *Pseudomonas flurescens.* ND-not detected

Values are mean (± standard error) (n=3) and values followed by the same letter in each column are not significantly different from each other as determined by DMRT (p á 0.05).

of the plant associated bacteria were able to produce a variety of plant growth promoting substances such as IAA, GA and siderophore in considerable amounts apart from diazotrophy.

Conclusion:

In this paper results from screening of naturally occurring diazotrophic bacteria from both wild and cultivated rice were discussed. The search for potential free-living diazotrophs and their diversity in nature is still a fascinating ongoing research and there is much work to be done to harness the whole potential of diverse diazotrophic bacterial communities in soil and their interaction with plants. The results of this study provide evidence for the presence of free-living diazotrophic bacteria and their growth promoting activities in both wild and cultivated rice variety. The addition to further studies that assessment of nifH, and 16S rRNA phylogenetic analyses need to identify the diazotrophic bacteria. The knowledge on the diversity of diazotrophic bacteria is required not only for understanding their ecological importance in the paddy soils, but also for their utilization in sustainable agricultural as inoculants of rice.

LITERATURE CITED

Bagnasco, P., De La Fuente, L., Gualtieri, G., Noya, F. and Anas, A. (1998). Fluorescent *Pseudomonas* spp. as biocontrol agents against forage legume root pathogenic fungi. *Soil Biol. Biochem.,* **30**: 1317-1322.

Beneduzi, A., Peres, D., Vargas, L.K., Bodanese-Zanettini, M.H. and Passaglia, L.M.P. (2008). Evaluation of genetic diversity and plant growth promoting activities of nitrogen-fixing Bacilli isolated from rice fields in South Brazil. *Appl. Soil Ecol.,* **39**: 311-320.

Borrow, A., Brian, P.W., Chester, V.E., Curtis, P.J., Hemming, H.G., Henehan, C., Jeffreys, E.G., Lloyd, P.B., Nixon, I.S., Norris, G.L.F. and Radley, M. (1955). Gibberellic acid, a metabolic product of the fungus *Gibberella fujikuroi*: some observations on its production and isolation. *J. Sci. Food agric.,* **6**: 340-348.

Bunt, J.S. and Rovira, A.D. (1955). Microbiological studies of some sub-antartic soils. *J. Soil. Sci.,* **6**:119-128.

Burris, R.H. (1972). Nitrogen fixation - assay methods and techniques. In: *Methods in enzymology*. (Eds.) Colowick, S.P. and N.O. Kaplan. Academic press, NEW YORK (U.S.A.), pp. 415- 431.

Burris, R.H. and Wilson, P.W. (1972). Methods for measurement of nitrogen fixation. In: *Methods in enzymology*. (Eds.) Colowick, S.P. and N.O. Kaplan. Academic press, NEW YORK (U.S.A.). pp. 355-367.

Cappuccino, J.C. and Sherman, N. (1992). Negative staining. In: *Microbiology*: *A laboratory manual*, (Eds.). Cappuccino, J.C., N. Sherman and C. Benjamin. Redwood City, pp.125-179.

Chandramohan, D. and Mahadevan, A. (1968). Indole acetic acid metabolism in soils. *Curr. Sci.,* **37**: 112-113.

Dubey, R.C. and Maheshwari, D.K. (2004). *Practical microbiology*. S. Chand and company Ltd., NEW DELHI (INDIA).

Fujii, T., Huang, Y.D., Higashitani, A., Nishimura, Y., Iyama, S., Hirota, Y., Yoneyama, Y. and Dixon, R.A. (1987). Effect of inoculation with *Klebsiella oxytoca* and *Enterobacter cloacea* on dinitrogen fixation by rice-bacteria associations. *Plant Soil,* **103**: 221-226.

Hossain, M. and Fischer K.S. (1995). Rice research for food security and sustainable agricultural development in Asia: Achievements and future challenges. *Geo. J.,* **35**: 286–295.

Humphries, E.C. (1956). Mineral composition and ash analysis. In: *Modern methods of plant analysis*. (Eds.) Peach, K. and M.V. Trecy. Springer Verlag, BERLIN (GERMANY). pp. 468-502.

Katznelson, H. and Bose, B. **(1959).** Metabolic activity and phosphate-dissolving capability of bacterial isolates from wheat roots, rhizosphere and non-rhizosphere soil. *Canadian J. Microbiol.,* **5**: 79–85.

Kirchhof, G., Reis, V.M., Baldani, J.I., Eckert, B., Döbereiner, J. and Hartmann, A. (1997). Occurrence, physiological and molecular analysis of endophytic diazotrophic bacteria in gramineous energy plants. *Plant Soil.,* **194**: 45–55.

Ladha, J.K., Barraquio, W.L. and Watanabe, I. (1983) Isolation and identification of nitrogen-fixing *Enterobacter cloacae* and *Klebsiella panticola* associated with rice plants. *Canadian J. Microbial.,* **29**: 1301-1308.

Lowry, O.H., Rosebrough, J.J., Farr, A.L. and R.L. Randall. (1951). Protein measurements with folin phenol reagent. *J. Biol. Chem.,* **193**: 265-273.

Lu, J.J. and Chang, T. (1980). Rice in its temporal and spatial perspectives. In: *Rice:Production and utilization*, Ed. B.S. Luh, p. 1-74, Avi Publishing, Westport, NEW ZEALAND (U.S.A.).

Mortenson, L.E. (1951). A simple method for measuring nitrogen fixation by cell-free enzyme preparations of *Clostridium pasteurianum. Analytical Biochem.,* **2**(3): 216-220.

Park, M., Kim, C., Yang, J., Lee, H., Shin, W., Kim, S. and Sa, T.M. (2005). Isolation and characterization of diazotrophic growth promoting bacteria from rhizosphere of agricultural crops of Korea. *Microbiol. Res.,* **160**:127–133.

Piao, Z., Cui, Z., Yin, B., Hu, J., Zhou, C., Xie, G., Su, B. and Yin, S. (2005). Changes in acetylene reduction activities and effects of inoculated rhizosphere nitrogen-fixing bacteria on rice. *Biol Fertil Soils.,* **41**: 371–378.

Pramer, D. and Schmidt, E.L. (1966). *Experimental soil microbiology*. Burgess Publ. Co., Minn., Minneopolis. 107 pp.

Reis, V.M., Olivares, FI. and Dobereiner, J. (1994) Improved methodology for isolation of *Acetobacter diazotrophicus* and confirmation of its endophytic habitat. *World J. Microbiol. Biotechnol.,* **10**: 101–104.

Roger, P.A. and Ladha, J.K. (1992). Biological N_2 fixation in wetland rice fields: Estimation and contribution to nitrogen balance. *Plant Soil,* **141**: 41–55.

Sato, Y.I. (1994). Ecological-genetic studies on wild and cultivated rice in tropical Asia. *Tropics,* **3**: 189-245.

Sgroy, V., Cassan, F., Masciarelli, M.O., Papa, F.D., Lagares, A. and Luna, V. (2009). Isolation and characterization of endophytic plant growth promoting or stress-homeostasis regulating bacteria associated to the halophyte *Prosopis strombulifera*. *Appl. Microbiol. Biotechnol.,* **7**: 341-346.

You, C. and Zhou, F. (1989). Non-nodular endorhizospheric nitrogen fixation in wetland rice. *Canadian J. Microbiol.,* **35**: 403-408