academicJournals

Vol. 9(11), pp. 814-820, 18 March, 2015
DOI: 10.5897/AJMR2013.6524
Article Number: A81400251650
ISSN 1996-0808

http://www.academicjournals.org/AJMR

African Journal of Microbiology Research

Full Length Research Paper

Isolation of potential phototrophic purple non-sulphur bacteria in paddy and their effects on paddy seedlings in hydroponic culture

Pavitra, B. V*, Sreenivasa, M. N, V. P Savalgi and Geetha Shirnalli

Department of Agricultural Microbiology, University of Agricultural Sciences, Dharwad, Karnataka, India.

Received 23 November, 2013; Accepted 8 January, 2014

In order to promote the growth of photosynthetic bacteria (PB) use as biofertilizers in paddy fields, this study conducted during 2011 to 2012 at Department of Agricultural Microbiology, University of Agricultural Sciences, Dharwad, Karnataka, India. Twenty phototrophic purple non-sulphur bacterial (PPNSB) isolates were isolated from paddy rhizosphere soils of Northern Karnataka. Isolates were tested by morphologically, biochemically and physiologically according in to Bergey's manual. All the isolates belong to PPNSB *Rhodobacter* sp. *In vitro* nitrogen fixation was estimated by percent nitrogen content in cells which were grown on nitrogen free culture broth. Maximum percent nitrogen content was recorded in culture broth inoculated with isolate NKPRSR -1 (2.50%). Based on percent nitrogen content, the best five efficient isolates were selected for hydroponic culture to see the effect on paddy seedlings growth under laboratory condition. In hydroponics Complete Randomized Design was used to accommodate 13 treatments with 5 replication, with the combination of nitrogen source and without nitrogen sources. Results revealed that treatment having isolate *Rhodobacter* sp. NKPRSR 1+ Nitrogen source show maximum plant height (26.67 cm), root length (13.17 cm), dry weight (16.50 mg/ plant) and percent nitrogen content (0.99%) after 30 days of sowing.

Key words: Isolation, purple nonsulphur bacteria, paddy, hydroponics, rhodobacter, rhizosphere.

INTRODUCTION

Purple phototrophic non-sulfur bacteria (PPNSB) constitute a diverse group among the anoxygenic phototrophic bacteria (APB) with a versatile metabolism (Imhoff, 1995) with potential for various biotechnological applications (Sasikala and Ramana, 1995a, b). In nature, they occur in aquatic as well as terrestrial environments where light of sufficient quantity and quality is available and partial anaerobic conditions prevail (Drews and Imhoff, 1991).

Rice (*Oryza sativa* L.) is a major world crop and more than half of the world's population is dependent on it. Rice crop needs more nitrogenous fertilizer, these chemical nitrogenous fertilizers are the most costly input for the production of rice crop and also they are not ecofriendly. Different ways of reducing nitrogenous fertilizer use in rice cultivation were sorted, while maintaining or enhancing crop output is desirable from both economic and environmental perspectives (Roger and Ladha, 1992). Increasing biological nitrogen fixation (BNF) associated with rice plants is an attractive approach to raise crop yield and reduce nitrogen fertilizer requirements.

*Corresponding author. E-mail: pavithramic9@gmail.com.

The most-studied microbial groups responsible for BNF in flooded rice fields are free-living cyanobacteria, heterotrophic bacteria and symbiotic cyanobacteria associated with the fern Azolla (Choudhury and Kennedy, 2004). In contrast, little attention has been devoted to the N_2-fixing phototrophic purple non-sulfur bacteria (PPNSB). Anoxygenic phototrophic bacteria are the major groups of microorganisms existing in paddy soils and contribute significantly to soil fertility (Habte and Alexander, 1980). Elbadry et al. (1999) have demonstrated beneficial effect of *Rhodobacter capsulatus* on rice variety in hydroponic cultures.

Kobayashi and Haque (1971) suggested a possible growth-promoting role of phototrophic purple non-sulphur bacteria (PPNSB) in rice fields. Maudinas et al. (1981) showed that in the absence of chemical nitrogen (Ammonium chloride), but in the presence of the diazotrophs *Azotobacter vinelandii* and *Rhodopseudomonas capsulatus* (now *Rhodobacter capsulatus*) in a liquid medium, rice plants can benefit from germination up to ear stage. Eldin and Elbanna (2011) also showed field evidence for the potential of *Rhodobacter capsulatus* as biofertilizer for flooded rice. So far, not much work has been carried out in India on PPNSB biofertilizer, therefore, this work is first of its kind aimed to study the effect of potential PPNSB isolates on rice seedling development in laboratory condition.

MATERIALS AND METHODS

Isolation

The paddy rhizosphere soil at tillering stage was collected from different rice growing districts of North Karnataka. For isolation enrichment culture technique followed as described by Archana et al. (2004) paraffin wax-overlay of pour plate, modified Biebl and Pfennig's (1981) (BP) medium of the following composition was used for plating the soil samples and for culturing the isolates (g $^{-}$1); KH_2PO_4: 0.5; $MgSO_4$, $7H_2O$: 0.2; NaCl: 0.4; NH_4Cl: 1.2; $CaCl_2$, $2H_2O$: 0.05; Yeast Extract: 0.3; Organic acids: 3.0 (Malate, Succinate and Pyruvate-1.0 g each); Ferric-Citrate (0.1% w/v) : 5 ml; Trace salt solution [(mg^{-} 1): HCl (25% v/v)-1 ml; $ZnCl_2$ 70; $MnCl_2$, $4H_2O$ 100; H_3BO_3 60; $CoCl_2$, $6H_2O$ 200; $CuCl_2$, H_2O 20; $NiCl_2$, $6H_2O$ 20; $NaMoO_4$, $2H_2O$ 40]: 1 ml; Agar:20 pH of the medium: 6.8 to 7.0 before autoclaving. In this study, in order to isolate nitrogen fixing isolates from the samples nitrogen compound was vomited from the media composition.

Approximately a gram of paddy soil was suspended in 10 ml saline water (0.7% NaCl w/v), mixed by overtaxing and used for subsequent tenfold dilutions in the saline water. Five hundred microlitres of the dilutions, used as inoculums, were pour plated with 20 ml of Biebl and Pfennig's (1981) agar medium (40 to 45°C) and the medium was allowed to solidify. The plates were then overlayed with molten paraffin wax (55 to 60°C; solidifies immediately on pouring over the agar). The plates were rotated gently in a circular motion while pouring the wax in order to spread it evenly over the agar surface (keep the plates open for a period of 10 min after pouring the paraffin wax in order to radiate the heat of the wax before closing the lid) and incubated at a temperature of 30 ± 2°C with the agar side of the plate exposed to a light intensity of 2400 lux. Following the development of colonies, the overlying paraffin wax was gently removed with a scalpel, colonies embedded in the agar sectioned out as blocks, transferred aseptically into 15 to 125 mm screw cap tubes fully filled with modified Biebl and Pfennig's (1981) liquid medium and the tubes incubated under a light intensity of 2400 lux at a temperature of 30 ± 2°C for 3 to 5 days. The cultured isolate was purified by repeated streaking on modified Biebl and Pfennig's (1981) agar slant prepared in 25 to 150 mm test tube. The pure colony obtained was transferred aseptically into a screw cap tube completely filled with Biebl and Pfennig's broth.

Physiological and biochemical characters tested according to Bergey's manual of determinative bacteriology after confirming the isolates belongs to PPNSB group (Imhoff and Truper, 1989). Cells grown in nitrogen free medium after 10 days of incubation were subjected for quantitative estimation of the amount of nitrogen fixed in the broth culture by Microkjeldahl method of Bremner and Mulvaney (1982). To the 10 ml of broth culture, 5 ml of concentrated H_2SO_4 and 200 mg catalyst mixture (potassium sulphate, copper sulphate and selenium in the ratio of 10:1:0.1) were added and allowed for digestion in block digester for 2 h to get clear digest. The clear digest was cooled and diluted with distilled water up to 10 ml. This was distilled in a distillation unit after addition of 20 ml of 40% sodium hydroxide solution to make the digest alkaline, in a Parnas-Wayner type distillation unit. The evolved ammonia was absorbed in four percent boric acid with mixed indicator and finally titrated with 0.05 N H_2SO_4 for colour change from green to red. From the volume of acid consumed, total nitrogen content was calculated.

Hydroponics study

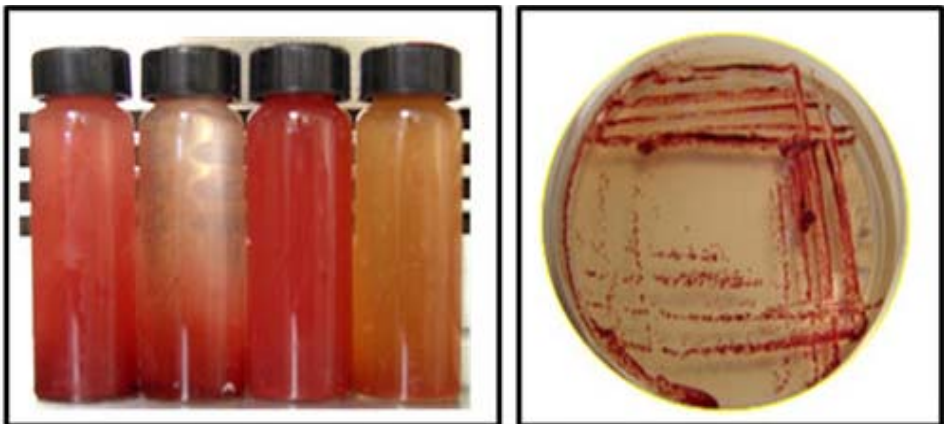
Isolated PPNSB isolates used for seeing the effect on rice seedling procedure was used for hydroponic culture according to Elbadry et al. (1999).

Rice seeds

Rice (*Oryza sativa* L.) seeds of Ahilasha variety brought from Aerial Rice Research Station (ARS) Mugad, Dharwad, Karnataka, India.

Microorganism

Five strains of the phototrophic purple non-sulfur bacterium *Rhodobacter* sp. isolates were used in the inoculation experiments. Bacterial inoculums were prepared immediately before inoculation. *Rhodobacter* sp. based on total nitrogen estimation selected five best isolates and reference strain *Rhodobacter capsulatus* KU002 was brought from Hydrabad Department of Biochemistry, MG University, Nalgonda. All the selected isolates were grown photosynthetically on Biebl and Pfennig's medium (1981). The cells were collected at the exponential growth phase by centrifugation at 10,000 rpm for 10 min and the cell pellets were washed twice with sterilized distilled water. The cells were resuspended in a medium having the same composition as the growth medium except that of NH_4Cl .The cultures were grown in completely filled glass bottles of 1000 ml capacity and incubated by degassing and using nitrogen. The logarithmic growth phase cells were centrifuged and the cell pellets were washed twice with sterilized distilled water and then resuspended in phosphate buffer 0.05 M, pH = 7. Here after these starved bacterial cells will be referred to as inoculums. The inoculums contained about 3.3 - 10^8 cells/ml.



(a) (b)

Figure 1. Appearance of pure culture of the isolates *Rhodobacter* sp. NKPRSR-1 (a) Cell suspension (b) Colony.

Rice nutrient solution (Heulin et al., 1987)

Solution A. ($g^{-}L$): $ZnSO_4 7H_2O$, 0.42; $MnSO_4 H_2O$, 1.30; $Na_2MoO_4 2H_2O$, 0.75; H_3BO_3, 2.8; $CuSO_4 7H_2O$, 0.026; $CoSO_4 7H_2O$, 0.07.

Solution B. ($g^{-}L$): $MgSO_4$, $7H_2O$, 2.00; $CaCl_2$, $2H_2O$, 2.00; $FeSO_4 7H_2O$, 0.44; EDTA, 0.40; Solution A, 20 ml. Solution C. ($g^{-}L$): K_2HPO_4, 90; KH_2PO_4, 60. Final nutrient solution: Solution B, 50 ml; Solution C, 15 ml; distilled water, 1000 ml. For N-treatments, the final nutrient solution was supplemented with 40 ppm nitrogen as NH_4Cl.

Seedling growth unit

The seedling growth unit used in the laboratory inoculation experiment is made of two parts. The upper part (A) is a plastic cup of 7 cm diameter and 9.2 cm height with a pored bottom. This part supports the germinating seeds and the aerial parts of the rice seedling. The lower part (B) is a 650 ml plastic bottle, with a mouth larger than the base of the plastic cup. At the start of the experiment, the two parts of the unit were sterilized separately using acetone and 70% ethanol. Thereafter, the plastic cup was tightly placed over the mouth of the plastic bottles, so that the solution level reached the rice plants continuously. The germinated rice seeds were transferred aseptically to the upper part, one seed for each hole.

Plant experiment

In this experiment, Abhilasha rice variety was used. Rice seeds of approximately similar size were surface sterilized with a 0.1% mercuric chloride solution for 1 min; followed by washing thoroughly with several changes of sterile distilled water (Yoshida et al., 1976) and allowed to germinate on nutrient agar in Petri dishes for 3 days at 30°C in the dark. Contaminant-free uniformly germinated seeds were aseptically transferred to sterilized growth assemblies containing 600 ml of the sterile nutrient solution with or without nitrogen. Nutrient solutions inoculated immediately with 60 ml prepared inoculums to yield approximately 3.3 - 10^8 cells/ml. The final volume of the solutions in the growth assembly was sufficient to cover the rice seeds.

The experiment comprised 13 treatments

First set

(1) Nitrogen-free nutrient solution + isolates 1, 2, 3, 4, 5 and ref. strain

Second set

(2) With nitrogen nutrient solution + isolates 1, 2, 3, 4, 5 and ref. strain

Uninoculated control

(3) Only nutrient solution with nitrogen

Five replicate assemblies were used and for each, treatment design used was Completely Rondomized Design (CRD). The plants were grown in a cabinet under natural light conditions at 29°C at day and 20°C at night. Measurements of the growth parameters of rice seedlings were recorded on 15th and 30th days after sowing. At the end of the 30th day experimental period, the plants were removed gently from the assemblies and washed in distilled water, the shoot and root portions were separated and the growth measurements were taken. All the plants in each assembly were taken together for measurements of dry weight (DW) and all the plants in each treatment were pooled for nitrogen determination by the Micro Kjeldohol technique. Statistical analysis data obtained from the different treatments were subjected to analysis of variance. Multiple mean comparisons were made by Duncan Multiple Range Tests using MSTAT- C software.

RESULTS AND DISCUSSION

In this study, totally 20 isolates were isolated and characterized according to their morphology, pigmentation and photo assimilated substrates. The cells of these isolates were rod to ovoid shaped all of the strains were Gram negative and motile. Red cell suspensions were formed by reddish brown colour colonies (Figure 1).

Table 1. Morphological, biochemical and physiological characteristics of the isolated purple non-sulphur bacteria in paddy PPNSB strains 1 to 20.

Characteristic	1	2	3	4	5	6	7	8	9	10	11	12	13	14	16	17	18	19	20
G. stain	G. N	G. N	G. N	G.N	G.N	G.N	G.N	G.N	G. N	G. N	G. N	G.N	G.N	G.N	G.N	G.N	G.N	G.N	G.N
Cell shape	rod	rod	rod	oval	rod	rod	rod	oval	rod	rod	rod	oval	rod	oval	oval	rod	rod	oval	oval
Motility	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Colour	Brownish	red	brown	red	brown	brown	brown	brown	Purplish	brown	red	red	red	red	red	brown	red	red	red
Slime production	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Nacl. Rec.	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
pH	7.0	7.5	7.0	8.0	7.5	7.0	7.0	7.0	7.0	7.0	7.0	7.0	7.0	6.5	6.5	7.0	7.5	7.5	7.5
Temperature	30	29	30	30	30	30	28	29	30	30	28	30	30	30	30	30	30	30	30
Growth on N free media	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Vitamins rec.	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Carbon source																			
Formate	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Acetate	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Propionate	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Tartarate	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Malate	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Fumarate	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Citrate	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Glucose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Monnitol	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Mannose	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Glycerol	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Gluconate	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Pyruvate	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Sucinate	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Absorption spectral(nm)	865	865	850	860	850	860	855	860	850	865	860	850	850	865	850	860	865	850	850

All the isolates grew well on neutral pH (6.8 to 7.5), optimum temperature (28 to 30°C) and isolates required both NaCl (0.2 to 0.4) and vitamins (Biotin). The photoheterotrophic growth of the isolates was tested on different organic carbon sources (Table 1). All the isolates inability to assimilate tartarate, citrate, glutamate, glycerol, mannitol and mannose they were shown luxuriant growth in rest of the carbon sources which were used in the study. Morphologically, one important characteristic of the PNSAB is the reddish pigmentation which indicated the presence of thephotosynthetic pigments (carotenoids and bacteriochlorophyll. The photosynthetic pigment present in PPNSAB could be bacteriochlorophyll *a*, *b* or both. The isolated bacteria in this study showed the characteristic reddish bloom under partial anoxy-photosynthetic conditions and the presence of only bacterichlotophyll *a* was observed (850 to 865 nm), (Braun and Scherz, 1990). Results were confirmed with Chen et al. (2003)

Table 2. Total percent nitrogen content in N free medium grown cells of PPNSB isolates *Rhodobacter sp.* isolated from paddy soils.

S. no	Isolates	Per cent nitrogen (%)
1	NKPRSR1	2.50[a]
2	NKPRSR2	2.33[ab]
3	NKPRSR3	1.93[bc]
4	NKPRSR4	2.00[abc]
5	NKPRSR5	1.75[c]
6	NKPRSR6	0.35[d]
7	NKPRSR7	0.39[d]
8	NKPRSR8	0.32[d]
9	NKPRSR9	0.30[d]
10	NKPRSR10	0.30[d]
11	NKPRSR11	0.29[d]
12	NKPRSR12	0.23[d]
13	NKPRSR13	0.32[d]
14	NKPRSR14	0.34[d]
15	NKPRSR15	0.26[d]
16	NKPRSR16	0.26[d]
17	NKPRSR17	0.28[d]
18	NKPRSR18	0.16[d]
19	NKPRSR19	0.12[d]
20	NKPRSR20	0.28[d]
21	Ref. *R. capsulatus* KU005	1.43[abc]
	CD@0.01	0.48

who isolated and characterized purple non-sulphur bacteria in swine manure waste and Soto-Feliciano et al. (2010) Isolated and characterized the purple non-sulfur anoxyphototropic bacteria after confirmation of characteristics of the isolates, tentatively grouped to phototrophic purple non-sulphur bacteria *Rhodobacter* sp. and for different isolates we coded NKPRSR (North Karnataka Paddy Rhizosphere Soil Rhodobacter).

All the PPNSB isolates were subjected to grow on nitrogen free medium and *in vitro* nitrogen fixation was estimated by estimating total per cent nitrogen content results presented in Table 2. All the isolates were able to grow on Nitrogen free BP medium and Highest per cent Nitrogen was recorded in PPNSB *Rhodobacter* sp. coded NKPRSR-1(2.25 %) and NKPRSR- 2 (2.33 %). Based nitrogen estimation five efficient strains selected for hydroponic culture NKPRSR- 1(2.25 %), NKPRSR- 2 (2.33 %l), NKPRSR- 3(1.95 %), NKPRSR- 4 (2.00 %) and NKPRSR 5 (1.75 %). Effects of PPNSB *Rhodobacter* sp. inoculation on rice seedling development of rice variety Abhilasha in hydroponic culture results presented in Table 3. Maximum shoot, root length, dry weight and per cent nitrogen content in plant were recorded in the treatment having both nitrogen source and efficient *Rhodobacter* sp. isolate NKPRSR- 1. Root length of rice seedlings inoculated with NKPRSR-1 (12.83 cm) (I±N) were significantly longer as compared to the treatment having only inorganic nitrogen source (7.17 cm)) at the end of the 30th day experimental period. Our results similar with the Elderly and Elbanna (1999) findings on rice varieties in hydroponics inoculated with *Rhodobacter capsulatus*. Murt and Ladha (1988) showed that *Azospirillum* inoculation of rice under hydroponic conditions significantly increased the root length of rice in agreement with our results. Apart from nitrogen fixation findings several research article on PPNSB group bacterium *Rhodobacter* sp. able to produce growth hormone production like indole acetic acid (Mujahid et al, 2011; Rajasekhar et al, 1998) and cytokinine (Serdyuk et al, 1993). In the present work, it was observed that elongation of root and increased numbers of new roots were initiated from the crown. Tien et al (1979) found similar effects of *A. brasilense* and plant hormones on lateral root production and main root elongation by pearl millet. In hydroponics lower jar consists of nutrient solution and isolated organism PPNSB bacterium *Rhodobacter* sp. is enable to grow in absence of chemical nitrogen and also presence of chemical nitrogen. Environment provided in the jar was not fully aerobic; it was partially anaerobic meaning microaerophillic condition because lower jar mouth was completely closed with alumilium foil and para filim. So, PPNSB bacterium

Table 3. Effect of *Rhodobacter capsulatus* strains inoculation and nitrogen fertilization on shoot height, root length, dry weight and N% content of rice grown in hydroponic culture.

Treatment	15 DAS (cm)	30 DAS (cm)	30 DAS Root length(cm)	Dry wt. (mg/plant)	% N content
Isolate NKPRSR1	13.50^{ab}	26.67^{ab}	12.50^{ab}	14.17^{ac}	0.99^{a}
Isolate NKPRSR2	12.17^{bcd}	24.03^{ab}	11.50^{bc}	13.17^{bgde}	0.83^{abc}
Isolate NKPRSR3	11.00^{def}	22.50^{cdef}	9.83^{def}	12.17^{def}	0.72^{bc}
Isolate NKPRSR4	10.33^{ef}	21.60^{ef}	9.17^{de}	11.00^{fg}	0.58^{cd}
Isolate NKPRSR5	9.83^{f}	21.17^{f}	8.50^{f}	10.17^{g}	0.42^{d}
Rhodobacter capsulatus KUOO5	13.33^{abc}	25.17^{abc}	10.00^{de}	13.50^{bcd}	0.86^{ab}
Only nitrogen	14.00^{a}	25.00^{abcd}	7.17^{g}	12.17^{def}	0.88^{ab}
Isolate NKPRSR1+N	13.67^{a}	27.67^{a}	13.17^{a}	16.50^{a}	0.99^{abc}
Isolate NKPRSR2+ N	11.83^{cde}	24.50^{bcde}	12.83^{a}	14.67^{b}	0.75^{bc}
Isolate NKPRSR3+ N	11.50^{def}	22.00^{def}	10.00^{de}	12.50^{def}	0.72^{bc}
Isolate NKPRSR4+ N	11.17^{def}	21.00^{f}	9.50^{def}	12.00^{def}	0.67^{bc}
Isolate NKPRSR5+ N	11.75^{cde}	21.00^{f}	9.17^{ef}	11.50^{efg}	0.73^{bc}
Rhodobacter capsulatus KU002+ N	13.83^{a}	21.50^{f}	10.83^{cd}	11.67^{fgg}	0.90^{ab}
CD @0.01%	1.14	2.78	1.27	1.58	0.17

N- Nitrogen

can grow luxuriantly with the provided condition, this was confirmed from earlier findings Elbanna et al (1999) in soilless culture. Inoculation in the presence of nitrogen were considerably maximum than those found in the nitrogen-free treatments and uninoculated (only nitrogen source) treatment. Results also confirmed with the Elbanna et al. (1999) findings in hydroponic culture. Nitrogenase regulation was different in this organism compare to other nitrogen fixing microorganisms (Masepohl et al, 2002). Results clearly indicate that inoculation with PPNSB *Rhodobacter* sp. with the inorganic nitrogen source, gave good plant growth performance in terms of improved seedling growth, as indicated by increase in dry weight (DW) of the aerial part and shoot height compared to the uncirculated once. The pronounced increase in seedling, dry weight, nitrogen percentage in roots and shoots with observed results from the study PPNSB *Rhodobacter* sp. isolates could produce more ammonium even with the presence of inorganic nitrogen source and the rice plants could benefit from the inoculated Isolates; *Rhodobacter* sp. can also produce growth hormones which were having plant growth promotion activity. Based on others findings confirmation with this paper result it can be concluded that inoculation gives benefits to rice seedling growth that almost commensurate with the addition of 40 ppm of nitrogen (ammonium chloride). This result is in harmony with the findings of others (Lee et al, 1977; Maudinas et al, 1981; Elderly and Elbanna 1999; Ramchander et al, 2012). With all these supportive findings and obtained results, PPNSB group of bacterium definitely have plant growth promoting activity.

The promotion of satisfactory seedling development is an important stage in crop development, essential for achieving optimal populations, and in turn, maximum yield. Therefore, the results obtained have been highly encouraging and provide good grounds for conducting further trials in pot and field experiment to bring potential PPNSB isolates as potential biofertilizer for paddy ecosystem.

Conclusion

Phototrophic purple non-sulfur bacterium *Rhodobacter* sp. isolates benefits rice seedling (*Oryza sativa* L.) in terms of growth and development under laboratory conditions. To the knowledge of this paper, this is the first reported evidence in India to study the potentialities PPNSB isolates in hydroponic culture study. Through optimizing rice cultivation and bacterial inoculation, it is possible to obtain the promotion of satisfactory seedling development as an important stage in crop development, essential for achieving optimal populations, and in turn, maximally yields an increase in grain yield and a decrease in chemical nitrogenous fertilizer requirement by inoculation with PPNSB isolates. This bacterium has great potential for the development use as a biofertilizer in rice cultivation to sustain soil fertility and reduce application of chemical fertilizer in future days.

ACKNOWLEDGEMENT

The authors would like to thank Indian Council of Agricultural Sciences for providing senior research fellowship, University of Agricultural Sciences, Dharwad.

REFERENCES

Archana Ch, Sasikala ChV, Ramana K, Arunasri (2004). Paraffin wax-overlay of pour plate, a method for the isolation and enumeration of purple non-sulfur bacteria. J Microbiol. Methods 59:423-425.

Biebl H, Pfennig H (1981). Isolation of members of the family Rhodospirillaceae. In: Starr MP, Stolp H, Truper HG, Balows A, and Schlegel HG (Eds.) The Prokaryotes. Springer-Verlag, Berlin. pp. 267- 273.

Braun P, Scherz A (1990). Polypeptides and bacteriochlorophyll organization in the light-harvesting complex B850 of *Rhodobacter sphaeroides* R-26.1. Biochemistry 30: 5177-5184.

Bremner JM, Mulvaney CS (1982). Nitrogen-total. In: A L Page, R H Miller, DR Keeney (Eds). Methods of soil analysis, Part 2-Chemical and microbiological properties. Agronomy 9:595-522.

Chen T, Schulte DD, Koelsch RK, Parkhurst AM (2003). Characteristics of phototrophic and non phototrophic lagoons for swine manure. Trans. Am. Soc. Agric. Eng. 46:1285-1292.

Choudhury AT, Kennedy IR (2004). Prospects and potentials for systems of biological nitrogen fixation in sustainable rice production. Biol. Fertil. Soils 39:219-227.

Drews G, Imhoff JF (1991) Phototrophic purple bacteria. In: Shively, J.M., Barton, L.L. (Eds.), Variations in Autotrophic Life. Academic Press, London, pp. 51-97.

Elbadry M, El-Bassel A, Elbanna Kh (1999). Occurrence and dynamics of phototrophic purple non-sulfur bacteria compared with other asymbiotic nitrogen fixers in rice fields of Egypt. World J. Microbiol. Biotechnol. 15:359-362.

Elbadry M, Gamal-Eldin H, Elbanna K (1999). Effects of *Rhodobacter capsulatus* inoculation in combination with graded levels of nitrogen fertilizer on growth and yield of rice in pots and lysimeter experiments. World J. Microbiol. Biotechnol. 15:393-395.

Eldin H, Elbanna K (2011). Field Evidence for the Potential of *Rhodobacter capsulatus* as Biofertilizer for Flooded Rice. Curr. Microbiol. 62:391-395.

Habte M, Alexander M (1980). Nitrogen fixation by photosynthetic bacteria in lowland rice culture. Appl. Environ. Microbiol. 38:342-347.

Heulin T, Guckert A, Balandreau J (1987). Stimulation of root exudation of rice seedlings by Azospirillum strains: Carbon budgets under gnotobiotic conditions. Biol. Ferti. Soil 4:9-14.

Imhoff J (1995). Taxonomy and physiology of phototrophic purple bacteria and green sulfur bacteria. In: Blankenship, R.E., Madigan, M.T, Bauer, C.E. (Eds.), Anoxygenic Photosynthetic Bacteria. Kluwer Academic Publishers, Netherlands. pp. 1 -15.

Imhoff JF, Truper HG (1989). The purple nonsulfur bacteria In Bergey's Manual of Systematic Bacteriology vol 3. Editor J T Staley, M P Bryant, N Pfennig and J G Holt, Williams and Wilkins Co. Baltimore. pp 1658-1661

Kobayashi M, Haque MZ (1971). Contribution to nitrogen fixation and soil fertility by photosynthetic bacteria. Plant Soil (Special Volume):443-456.

Lee KK, Castro T, Yoshida T (1977). Nitrogen fixation throughout growth and varietal differences in nitrogen fixation by the rhizosphere of rice planted in pots. Plant Soil 48: 613-619.

Masepohl B, Drepper T, Paschen A, Klipp W (2002). Regulation of nitrogen fixation in the phototrophic purple bacterium *Rhodobacter capsulatus*. J. Mol. Microbial. Biotechnol. 4(3):243-248.

Maudinas B, Chemardin M, Yovanovitch E, Gadal P (1981). Gnotobiotic culture of rice plants up to ear stage in the absence of combined nitrogen source but in the presence of free living nitrogen fixing bacteria *Azotobacter vinelandii* and *Rhodopseudomonas capsulatus*. Plant Soil 60:85-97

Mujahid Md, Sasikala Ch., Ramana ChV (2011). Production of indole-3-acetic acid and related indole derivatives from L-tryptophan by *Rubrivivax benzoatilyticus* A2. Appl. Microbiol. Biotechnol. 89:1001-1008.

Rajasekhar N, Sasikala C, Ramana CV (1998). Photobiotransformation of indole to its value-added derivatives by *Rhodobacter sphaeroides* OU5 J. Ind. Microbiol. Biotechnol. 20: 177-179.

Ramchander M, Pratap R, Girisham S, Reddy SM (2012). Effect of bioinoculation of *Rhodobacter capsulatus* ku002 on two rice varieties of India. Inter. J. Appl. Biol. Pharm. Technol. 3(1):373-375.

Roger PA, Ladha JK (1992). Biological nitrogen fixation in wetland rice fields: estimation and contribution to nitrogen balance. Plant Soil 141:41-45.

Sasikala Ch, Ramana ChV (1995a). Biotechnological potentials of anoxygenic phototrophic bacteria: II. Biopolyesters, biopesticide, biofuel and biofertilizer. Adv. Appl. Microbiol. 41:227- 278.

Sasikala Ch, Ramana ChV (1995b). Biotechnological potentials of anoxygenic phototrophic bacteria: Production of single cell protein, vitamins, ubiquinones, hormones and enzymes and use in waste treatment. Adv. Appl. Microbiol. 41:173-226.

Serdyuk OP, Smolygiana LD, Kobzar EF, Gogotov IN (1993). Occurrence of plant hormones in cells of the phototrophic purple bacterium *Rhodospirillum rubrum* 1R. FEMS Microbiol. Lett. 109:113-116.

Soto-Feliciano K, De Jesús M,Vega- Sepúlveda J, Ríos-Velázquez C (2010). Isolation and characterization of purple non-sulfur anoxyphototropic bacteria from two microecosystems : tropical hypersaline microbial mats and bromeliads phytotelmata. Curr. Res. Technol. Edu.Topics Appl. Microbiol. Microb. Biotechnol. 1(2):109-116.

Yoshida S, Forno DA, Cock JH, Gomez KA (1976). Laboratory Manual for Physiological Studies of Rice. IRRI 3rd edn, Philippines.