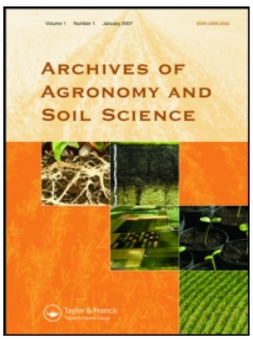

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Developing PGPR consortia using novel genera *Providencia* and *Alcaligenes* along with cyanobacteria for wheat

Mallappa Manjunath[a], Radha Prasanna[a]*, Pratima Sharma[a], Lata Nain[a] and Rajendra Singh[b]

[a]*Division of Microbiology, Indian Agricultural Research Institute (IARI), New Delhi, India;* [b]*National Phytotron Facility, Indian Agricultural Research Institute (IARI), New Delhi, India*

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A pot experiment was undertaken using unsterile soil supplemented with the recommended dose of chemical fertilizers in order to investigate the effect of indole-3-acetic acid (IAA)-producing proteo- and cyanobacterial strains on the growth and yield of wheat (*Triticum aestivum* variety PBW343). Two proteobacterial (WRB4 *Providencia* sp. and WRB10 *Alcaligenes* sp.) and two cyanobacterial (WRC3 *Anabaena oscillarioides* and WRC4 *Anabaena torulosa*) strains were used individually and in combination. The treatment in which proteobacteriumWRB4 (*Providencia* sp.) was inoculated showed significantly higher values in comparison with controls for various plant-growth parameters recorded, i.e. shoot length, root length, shoot weight, root weight and crop biomass, followed by the treatment in which the WRC3 (*Anabaena oscillarioides*) was used. A positive interaction among the proteo- and cyanobacterial strains, in particular WRC3 and WRB4, was also observed by way of enhancement of plant-growth parameters. Significant enhancement in soil microbiological activities such as fluorescein diacetate (FDA) hydrolysis and dehydrogenase activity were recorded in the treatments, particularly in those inoculated with cyanobacterial strains, when compared with fertilizer controls. This is a first-time report on the potential of selected combinations of proteobacterial genera such as *Providencia* and *Alcaligenes* and cyanobacteria such as *Anabaena* as plant growth-promoting organisms in wheat crop.

Keywords: *Anabaena* sp.; *Alcaligenes* sp.; plant growth promotion; *Providencia* sp.; rhizobacteria; wheat

Introduction

The search for microorganisms that improve soil fertility and enhance plant nutrition continues to attract attention due to the increasing cost of fertilizers and some of their negative environmental impacts (Bowen and Rovira 1999; Barea et al. 2004). Rhizobacteria with beneficial effects on plant growth and development are referred to as plant-growth-promoting rhizobacteria (PGPR). Exploitation of such plant-growth-promoting bacteria to improve crop production has become an integral part of sustainable agriculture. The isolation of microorganisms, screening for desirable characters and selection of efficient strains are important steps to

*Corresponding author. Email: radhapr@gmail.com

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optimize high crop yields and improve the sustainability of the ecosystem. PGPR influence plant growth directly by producing and releasing phytohormones or indirectly by limiting the deleterious effects of phytopathogenic organisms in the rhizosphere or facilitating the availability and uptake of certain nutrients from the root environment (Barea et al. 2004; Zahir et al. 2004). Selected strains of PGPR are used as seed inoculants (Sahin et al. 2004; Zahir et al. 2004) and the colonization of roots by bacteria having several beneficial traits has been shown to promote and stimulate plant growth and development (Sindhu et al.1999). Such bacteria have been applied to a wide range of agricultural crop species for the purpose of growth enhancement, including increased seed emergence, plant weight, crop yields and disease control (Kloepper et al. 1991). One of the phytohormones produced by soil microorganisms is indole-3-acetic acid (IAA) which is an important hormone for plant growth and development (Yasmin et al. 2009). The capacity to synthesize IAA is widespread among soil- and plant-associated bacteria. It has been estimated that 80% of bacteria isolated from the rhizosphere can produce the plant-growth regulator IAA (Patten and Glick 1996). IAA production has been reported in isolates of *Enterobacter, Pseudomonas, Azospirillum, Gluconacetobacter, Pseudomonas* and *Rhizobium* spp. (Koga et al. 1994; Pedraza et al. 2004). Many cyanobacteria such as *Anabaena, Nostoc*, *Cylindrospermum, Calothrix* and *Plectonema* are also capable of synthesizing IAA (Sergeeva et al. 2002; Prasanna et al. 2009, 2010). In an earlier study (data not shown), we screened a set of proteo- and cyanobacterial strains for their plant-growth-promoting traits under laboratory conditions in terms of biochemical attributes, including IAA production and plant-growth promotion using hydroponic experiments. Promising strains showing high levels of IAA production were selected for further experimentation. The objective of this study was to evaluate the plant-growth-promoting abilities of two proteobacterial and two cyanobacterial strains alone and in combination in wheat crop.

Materials and methods

Organisms

Two proteobacterial strains WRB4 (*Providencia* sp.) and WRB10 (*Alcaligenes* sp.) and two cyanobacterial strains WRC3 (*Anabaena oscillarioides*) and WRC4 (*Anabaena torulosa*) were selected on the basis of preliminary screening of a set of proteo- and cyanobacterial strains taken from the germplasm of Division of Microbiology, IARI, New Delhi. The proteobacterial cultures were grown and maintained in nutrient broth and on nutrient agar slants. Log-phase cultures of bacteria (48 h) incubated at 30 $\pm$ 2°C in a low temperature (BOD) incubator–shaker were utilized. Log-phase cyanobacterial cultures (6 d) were utilized after growth in nitrogen-free BG-11 media (Stanier et al. 1971). Growth conditions involved light/dark cycles (16:8 h) with white light (50–55 μmol photons m^{-2} s^{-1}) and temperatures of 28 $\pm$ 2°C in nitrogen-free BG-11 medium in Haffkine flasks.

Biochemical characterization of the proteobacterial and cyanobacterial strains

Fifty microlitres of homogenized cultures of two cyanobacterial strains and two proteobacterial strains were inoculated in the wells of HiCarbohydrate Kit^{TM} plates (HiMedia Laboratories, India). For cyanobacteria, incubation in 16:8 h (light/dark

cycles) and continuous dark conditions for 48 h was undertaken and colorimetric observations were recorded and evaluated according to the manufacturer's instructions.

Experimental set-up for the pot experiment

The two cyanobacterial strains, i.e. WRC3 (*Anabaena oscillarioides*) and WRC4 (*Anabaena torulosa*) grown in BG-11 medium were centrifuged at 3000 rpm for 30 min. The pellet was washed with sterile water to provide a chlorophyll content of 5.0 μg pot^{-1} for soaking wheat seeds in treatments (five seeds pot^{-1}) involving an individual strain/combination of strains.

The two rhizoproteobacterial strains, i.e. WRB4 (*Providencia* sp.) and WRB10 (*Alcaligenes* sp.), were grown overnight in a shaker in nutrient broth until an optical density (OD) $>$ 0.6 at 600 nm was reached. The cultures were centrifuged at 3000 rpm and the pellet was dissolved in sterile water and 10^4 cells seed^{-1} was used as an index for soaking wheat seeds (variety PBW343) in case of an individual strain and combination of strains, respectively. Treatments included inoculation with individual proteobacteria and cyanobacteria and a combination of bacteria/cyanobacteria or cyanobacterium + bacterium. Treatment details are given in Table 1.

The experiment was carried out in a glasshouse at the National Phytotron Facility, IARI, New Delhi. Plastic pots were filled with 12 kg soil of unsterile soil from IARI fields, which was of a sandy loam type with a pH of 6.8 $\pm$ 0.2. The soil was supplemented with the recommended level of nitrogen, phosphorus and potassium, i.e. 120 kg N in the form of urea, 60 kg P (P_2O_5) in the form of single super phosphate and 40 kg K_2O ha^{-1} in the form of muriate of potash, respectively, for wheat crop (*Triticum aestivum* variety PBW343) in accordance with the different treatments. The environmental conditions (26 and 20°C during day and night, respectively) were simulated as recommended for wheat crop. The experiment was undertaken up to the harvest stage, for a period of 130 days, and included 12 treatments with 4 replications including two controls of full-dose N, P and K and 2/3 N + full dose of P and K fertilizers (details are given in Table 1). Data were recorded for the following parameters: seed germination, root length, root weight, shoot length, shoot weight, crop biomass and grain weight.

Biometrical observations

Plant parameters such as crop biomass, shoot length, shoot weight, root length, root weight and grain weight were recorded at the time of harvesting. Vigour index was calculated using the formula (root length + shoot length) $\times$ germination percentage.

Soil parameters

The parameters given below were evaluated at the harvest stage of crop.

Fluorescein diacetate hydrolysis assay

The fluorescein diacetate (FDA) hydrolysis assay was carried out using 1 g soil suspended in 5 ml of potassium phosphate buffer (pH 7.6) and FDA (0.5 mg ml^{-1}). After incubation for 2 h at 37°C, the reaction was stopped using acetone. The solution was filtered through Whatman No. 1 filter paper and the absorbance of the

Table 1. Effect of inoculation of wheat with two bacterial (WRB4 and WRB 10) and two cyanobacterial (WRC3 and WRC 4) strains on various plant growth parameters.

Treatments	Conditions	Crop biomass (g pot^{-1})	Shoot length (cm)	Shoot weight (g)	Root length (cm)	Root weight (g)	Grain weight (g pot^{-1})	Vigour index
T1	WRC3 + 2/3 N + full dose of P and K	49.037*	58.944	9.147	10.944	0.942	5.183	6988.8
T2	WRC4 + 2/3 N + full dose of P and K	45.883	58.111	8.224	6.722	0.382	4.607	6483.3
T3	WRB4 + 2/3 N + full dose of P and K	49.297*	61.056	9.400*	12.556*	0.969*	5.640*	7361.2*
T4	WRB10 + 2/3 N + full dose of P and K	47.463	61.333	8.131	11.667	0.807	4.890	7300.0*
T5	WRC3 + WRB4 + 2/3 N + full dose of P and K	43.317	62.667*	9.144	10.889	0.918	4.897	7355.6*
T6	WRC3 + WRB10 + 2/3 N + full dose of P and K	41.927	61.500	4.686	10.722	0.832	4.333	7222.2*
T7	WRC4 + WRB4 + 2/3 N + full dose of P and K	43.590	57.333	4.513	10.722	0.846	4.863	6805.5
T8	WRC4 + WRB10 + 2/3 N + full dose of P and K	42.907	59.000	3.810	12.444*	0.817	3.637	7144.4*
T9	WRC3 + WRC4 + 2/3 N + full dose of P and K	35.297	52.556	3.411	9.778	0.802	3.537	6233.4
T10	WRB4 + WRB10 + 2/3 N + full dose of P and K	33.560	50.389	5.620	6.278	0.317	2.340	5666.7
T11	Full dose of NPK	46.900	61.556	8.236	10.444	0.899	4.673	7200.0*
T12	2/3 N + full dose P and K.	41.777	57.333	5.432	9.722	0.649	2.900	6705.0
SEM		0.263	0.290	0.058	0.169	0.007	0.071	66.588
CD @5%		0.725	0.800	0.160	0.466	0.019	0.195	183.782

*Significantly higher values.

SEM, standard error of mean; CD, critical difference; WRC3, cyanobacterial strain (*Anabaena oscillariodes*); WRC4, cyanobacterial strain (*Anabaena torulosa*); WRB4, proteobacterial strain (*Providencia*); WRB10, WRB4, proteobacterial strain (*Alcaligenes*).

supernatant taken at 490 nm using fluorescein standard (Green et al. 2006). The values were expressed as μg fluorescein released g^{-1} soil h^{-1}.

Dehydrogenase activity

Dehydrogenase activity was assayed using 6 g soil, incubated with 1 ml of triphenyl tetrazolium chloride (3%) for 24 h in the dark. Methanol was added to terminate the enzymatic reaction and the supernatant was filtered and absorbance taken at 485 nm (Casida et al. 1964). Values were expressed as μg of triphenyl formazan (TPF) g^{-1} day^{-1}.

Statistical analysis

Triplicate sets of data for the various parameters evaluated were subjected to analysis of variance (ANOVA) in accordance with the experimental design (completely randomized design) using MSTAT-C statistical package to quantify and evaluate the source of variation and undertake correlation analyses. Critical difference (CD) values were calculated at $p = 0.05$, and are represented as standard errors (SE) in the tables. Standard deviation (SD) values are depicted in the graphs as bars.

Results

Pot experiment

Treatment T3 (WRB4 *Providencia* sp.) recorded significantly higher values; followed by T1 in which WRC3 (*Anabaena oscillarioides*) was inoculated in terms of crop biomass. Shoot length was highest in T5 followed by T11, T6, T4 and T3. Treatment T3 recorded significantly higher shoot weight followed by T5 and T1. In terms of root weight and grain weight, treatment T3 recorded the highest values, whereas in terms of root length, treatments T3 and T8 recorded the highest values. Vigour index was highest in T3 followed by T5 and T4 (Table 1).

Fluorescein diacetate hydrolysis assay

The highest values for FDA hydrolysis were observed in T3 which was significantly higher than T1, T7 and T5. The lowest values were recorded in uninoculated controls, i.e. T11 and T12 (Figure 1a).

Dehydrogenase activity

Treatment T3 recorded the highest dehydrogenase activity followed by T1 and T5. The lowest values were recorded in uninoculated controls, i.e. T11 and T12 (Figure 1b). Correlation analyses between selected soil microbiological and biometrical parameters revealed a positive relationship (Figure 2).

Biochemical characterization of the proteobacterial and cyanobacterial strains

The proteobacterial strain WRB4 (*Providencia* sp.) was found to utilize substrates such as fructose, dextrose, galactose, sodium gluconate, glycerol, salicin, inositol, mannitol, adonitol, α-methyl-D-glucose, ribose, ornithine besides exhibiting the

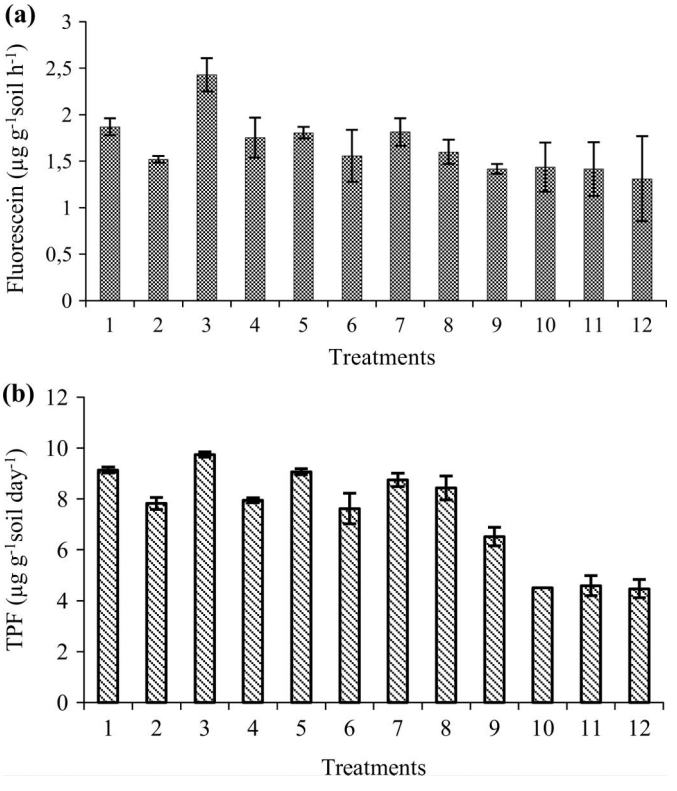

Figure 1. (a) Effect of plant-growth-promoting bacteria and cyanobacteria on fluorescein diacetate (FDA) activity ($\mu g\ g^{-1}$ soil h^{-1}). (b) Effect of plant-growth-promoting bacteria and cyanobacteria on dehydrogenase (TPF) activity ($\mu g\ g^{-1}$ soil day^{-1}).

ability of esculin hydrolysis, phenylalanine deamination, urease and H_2S production based on the colorimetric observations. The proteobacterial strain *Alcaligenes* sp. (WRB10) uniquely utilized only lysine, whereas both strains tested positive for citrate and malonate utilization and nitrate reduction. Under light conditions, the strain *Anabaena oscillarioide*s (WRC3) utilized fructose, trehalose, sucrose, mannose, inulin and malonate, and the strain *Anabaena torulosa* (WRC4) utilized galactose, rhamnose and D-arabinose. Both strains tested positive for utilization of xylose, maltose, dextrose, melibiose besides nitrate reduction. Under dark conditions, *Anabaena oscillarioides* utilized melezitose, citrate and nitrate. The strain *Anabaena torulosa* utilized malonate under dark, D-arabinose and citrate under both light and dark conditions. Both strains utilized citrate under dark conditions (Table 2).

The proteobacterial strains produced 14–26 $\mu g\ ml^{-1}$ IAA in the absence/presence of tryptophan under aerated conditions, respectively, and the cyanobacterial strains produced 8.0–11.0 $\mu g\ ml^{-1}$ in the absence/presence of tryptophan (Manjunath 2010, data submitted).

Discussion

The complexity of the soil system and its role in crop growth and productivity is determined by diverse interactions among the physical, chemical and biological

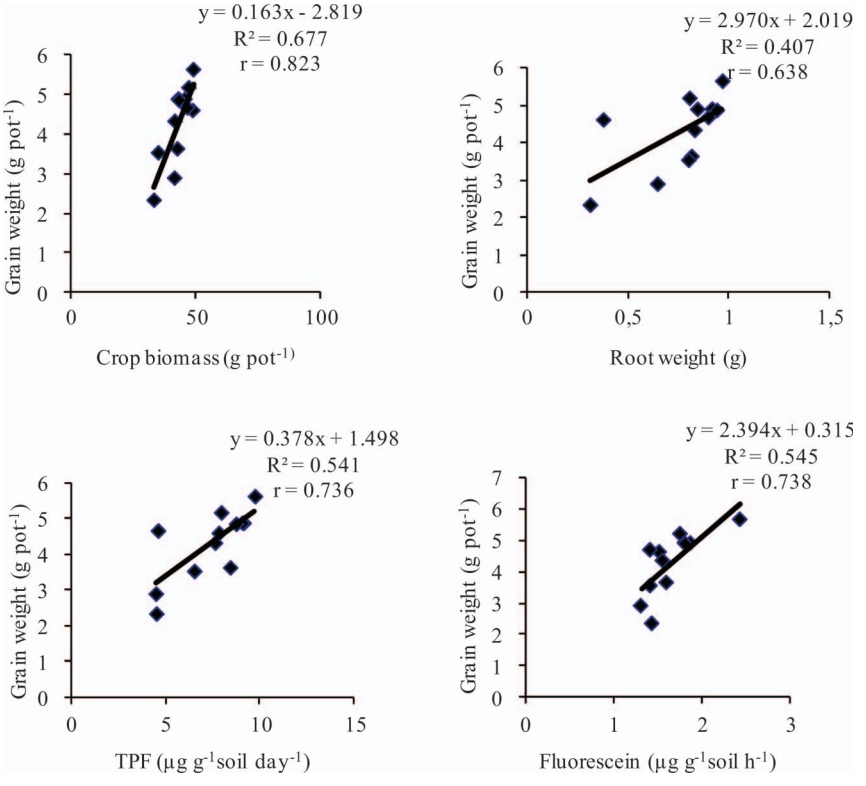

Figure 2. Correlation analyses between (a) crop biomass (g pot^{-1}) and grain weight (g pot^{-1}); (b) root weight (g) and grain weight (g); (c) TPF (μg g^{-1}soil day^{-1}) and grain weight (g pot^{-1}); (d) FDA (μg g^{-1}soil h^{-1}) and grain weight (g pot^{-1}).

components. Microbial activity in the rhizosphere affects not only root development and the supply of available nutrients to plants, but also indirectly influences the microfauna and flora through modifications of the quality and quantity of root exudates (Glick 1995; Barea et al. 2004). A significant increase in crop growth and yields has been demonstrated in important crops in response to inoculation with PGPR such as *Pseudomonas, Azospirillum, Azotobacter* and cyanobacteria (Kloepper et al. 1991; Glick and Bashan 1997; Karthikeyan et al. 2007; Prasanna et al. 2009).

Our aim was to evaluate a set of proteo- and cyanobacterial strains for their potential in enhancing plant growth and yield in a wheat (variety PBW343) crop. Preliminary screening of these cyanobacterial strains and rhizobacteria through an in vitro assay method employing a seed germination test on soft agar and their plant-growth-promoting activities under gnotobiotic conditions provided sufficient positive results regarding the effectiveness of these strains (Prasanna et al. 2009, 2010; Manjunath 2010). This prompted us to evaluate different combinations of the promising cyanobacteria with rhizobacteria in wheat crop under net house conditions with unsterile soil, as a prelude to developing effective consortia. The proteo- and cyanobacterial strains used in this study produce IAA in the presence

Table 2. Substrate utilization patterns of the bacterial (WRB4 and WRB10) and cyanobacterial ((WRC3 and WRC4) strains.

Substrate	Utilization pattern of		Utilization pattern of WRC3		Utilization pattern of WRC4	
	WRB4	WRB10	Light	Dark	Light	Dark
Lactose	−	−	−	−	−	−
Xylose	−	−	+	−	+	−
Maltose	−	−	+	−	+	−
Fructose	+	−	+	−	−	−
Dextrose	+	−	+	−	+	−
Galactose	+	−	−	−	+	−
Raffinose	−	−	−	−	−	−
Trehalose	−	−	+	−	−	−
Melibiose	−	−	+	−	+	−
Sucrose	−	−	+	−	−	−
L-Arabinose	−	−	−	−	−	−
Mannose	+	−	+	−	−	−
Inulin	−	−	+	−	−	−
Sodium gluconate	+	−	−	−	−	−
Glycerol	+	−	−	−	−	−
Salicin	+	−	−	−	−	−
Glucosamine	−	−	−	−	−	−
Dulcitol	−	−	−	−	−	−
Inositol	+	−	−	−	−	−
Sorbitol	−	−	−	−	−	−
Mannitol	+	−	−	−	−	−
Adonitol	+	−	−	−	−	−
α-Methyl-D-glucose	+	−	−	−	−	−
Ribose	+	−	−	−	−	−
Rhamnose	−	−	−	−	+	−
Cellulose	−	−	+	+	−	−
Melezitose	−	−	−	+	−	−
α-Methyl-D-mannoside	−	−	−	−	−	−
Xylitol	−	−	−	−	−	−
ONPG	−	−	−	−	−	−
Esculin hydrolysis	+	−	+	+	−	−
D-Arabinose	−	−	−	−	+	+
Citrate utilization	+	+	−	+	+	+
Malonate utilization	+	+	+	−	−	+
Sorbose	−	−	−	−	−	−
Lysine utilization	−	+	−	−	−	−
Ornithine utilization	+	−	−	−	−	−
Urease	+	−	−	−	−	−
Phenylalanine deamination	+	−	−	−	−	−
Nitrate reduction	+	+	+	+	+	−
H_2S production	+	−	−	−	−	−

and absence of tryptophan and had shown promise in hydroponic experiments with wheat seedlings (Manjunath 2010).

Seed treatment with two cyanobacterial strains and two proteobacterial strains, either individually or in combination, enhanced crop biomass, shoot length, shoot weight, root length, root weight, vigour index and grain weight over fertilizer

Table 3. Comparative performance of bacterial (WRB4 and WRB10)/cyanobacterial (WRC3 and WRC 4) strains in terms of plant parameters in the pot experiment.

Parameters evaluated	Top ranked treatments	Inclusion of strain in the top ranked treatments									
		WRB4	WRB10	WRC3	WRC4	WRC3 + WRB4	WRC3 + WRB10	WRC4 + WRB4	WRC4 + WRB10	WRC3 + WRC4	WRB4 + WRB10
Crop biomass	T3	+	−	−	−	−	−	−	−	−	−
	T1	−	−	+	−	−	−	−	−	−	−
	T4	−	+	−	+	−	−	−	−	−	−
	T11	−	−	−	−	−	−	−	−	−	−
	T7	−	−	−	−	−	−	+		−	−
Shoot length	T5	−	−	−	−	+	−	−	−	−	−
	T11	−	−	−	−	−	−	−	−	−	−
	T8	−	−	−	−	−	−	−	+	−	−
	T1	−	−	+	−	−	−	−	−	−	−
	T2	−	−	−	+	−	−	−	−	−	−
Shoot weight	T3	+	−	−	−	−	−	−	−	−	−
	T1	−	−	+	−	−	−	−	−	−	−
	T11	−	−	−	−	−	−	−	−	−	−
	T2	−	−	−	+	−	−	−	−	−	−
	T4	−	+	−	−	−	−	−	−	−	−
Root length	T3	+	−	−	−	−	−	−	−	−	−
	T8	−	−	−	−	+	−	−	+	−	−
	T4	−	+	−	−	−	−	−	−	−	−
	T1	−	−	+	−	−	−	−	−	−	−
	T5	−	−	−	−	+	−	−	−	−	−
Root weight	T3	+	−	−	−	−	−	−	−	−	−
	T1	−	−	+	−	−	−	−	−	−	−
	T11	−	−	−	−	−	−	−	−	−	−
	T7	−	+	−	−	−	−	+	−	−	−
	T6	−	−	−	−	+	+	−	−	−	−

(*continued*)

Table 3. (*Continued*).

Parameters evaluated	Top ranked treatments	Inclusion of strain in the top ranked treatments									
		WRB4	WRB10	WRC3	WRC4	WRC3 + WRB4	WRC3 + WRB10	WRC4 + WRB4	WRC4 + WRB10	WRC3 + WRC4	WRB4 + WRB10
Grain weight	T3	+	−	−	−	−	−	−	−	−	−
	T1	−	−	+	−	−	−	−	−	−	−
	T5	−	−	−	−	+	−	−	−	−	−
	T4	−	+	−	−	−	−	−	−	−	−
	T2	−	−	−	+	−	−	−	−	−	−
Vigour index	T3	+	−	−	−	−	−	−	−	−	−
	T5	−	−	−	−	+	−	−	−	−	−
	T11	−	−	−	−	−	−	−	−	−	−
	T7	−	−	−	−	−	−	+		−	−
	T1	−	−	+	−	−	−	−	−	−	−
Total		6	5	7	4	6	1	3	2	−	−

controls. Treatment T3 (*Providencia* sp.) recorded the highest grain weight which was 20.69% higher than fertilizer controls. Increase in root weight, shoot weight and yield of spring wheat upon inoculation with the PGPR *Azospirillum brasilense* was reported by Gravel et al. (2007) and Shaharoona et al. (2006). Abbas and Okon (1993) reported that increased growth observed in canola, tomato and wheat was due to IAA and other plant hormones produced by *Azotobacter paspali* in nonsterile soil. In addition to IAA, bacteria such as *Paenibacillus polymyxa* and *Azospirilla* also release other compounds in the rhizosphere that could indirectly contribute to plant growth promotion like indole-3-butyric acid and tryptophol or indole-3-ethanol (El-Khawas and Adachi 1999). A similar increase in vigour index and other plant parameters by rhizobacteria has been reported in wheat and other cereals like sorghum and pearl millet (Niranjan et al. 2004; Shaukat et al. 2006). A significant increase in seedling vigour may occur due to greater synthesis of auxins, particularly IAA (Bharathi et al. 2004). Our earlier studies revealed that the strains used in this investigation produced significant amounts of IAA (Manjunath 2010; data submitted elsewhere).

The genus *Providencia* belongs to the Enterobacteriaceae, is a nonspore-forming Gram-negative bacillus and has mainly been investigated for its role in animal and human infections, however, reports on the role of *Providencia* in bioremediation, especially of pesticides such as chlorpyriphos (Van Hamme et al. 2003; Surekha Rani et al. 2008), and in phosphate solubilization are also available (Rodriguez and Fraga 1999). In our earlier study, WRB4 (*Providencia sp.*) had shown IAA production and enhanced seed germination (data submitted elsewhere) and in the current investigation brought about significantly higher plant-growth promotion of wheat, which indicated its potential as an inoculant. The strain tested negative when checked clinically for pathogenicity using a DNAse test (Manjunath 2010).

In an earlier study (Lin et al. 2000), *Alcaligenes faecalis* was shown to be a key organism for use as an inoculant, either singly or in mixtures, for rice crop (You et al. 1991). However, its role in wheat crop has not been reported. In our study, the strain (WRB10, *Alcaligenes sp.*) performed well when used individually or in combination (T4 and T6) and needs to be investigated for its performance along with other PGPR strains for developing effective consortia.

Earlier studies have revealed that higher crop biomass can be correlated with IAA production, as in the case of *Azospirillum lipoferum* DSM 1691, in unsterile soil (Nezarat and Gholami 2009). Our results showed a similar trend, i.e. the highest crop biomass was recorded in treatment T3 (*Providencia* sp.) followed by T1 (*Anabaena oscillarioides*). Çakmakçı et al. (2006, 2007) reported that inoculation with plant growth-promoting rhizoproteobacteria enhanced seedling growth and shoot weight of wheat plants significantly compared with controls; the maximum root and shoot weights in wheat were found in *Paenibacillus polymyxa* RC05 followed by *Pseudomonas putida* RC06 and *Bacillus megaterium* RC07. Nezarat and Gholami (2009) also reported that inoculation of maize seeds with proteobacterial strains significantly increased plant height (14.3–21.7%). Interestingly, in our experiment, maximum shoot length was observed in treatment T5, which involved a combination of *Providencia sp.* and *Anabaena oscillarioides* (Table 1 and Supplementary Table 1 – available online only). Although the combination of proteobacterial strains has been reported to improve plant growth and yields, reports on combination of proteobacterial and cyanobacterial strains are scarce.

Cyanobacterial strains have been reported to produce extracellular amino acids such as aspartic acid, glutamic acid, proline, valine, glycine and alanine, besides

phytohormones such as IAA (Sergeeva et al. 2002; Karthikeyan et al. 2009; Prasanna et al. 2010) at various stages of growth. In our study, both *Anabaena* strains, in particular WRC3 *Anabaena oscillaroides*, performed significantly better than control treatments, alone and in combination with *Providencia* sp. and *Alcaligenes* sp. Synergistic effects were also recorded among the cyano- and proteobacterial strains in enhancing plant growth and microbial activity of soil (Supplementary Table 1 – available online only). This may be indicative of metabolite production by cyanobacterial strains, which not only improve plant growth, but also bring about qualitative and quantitative changes in microbiological populations in soil.

Dehydrogenase activity is known to increase markedly with increasingly active viable cells (Casida et al. 1964). In an earlier study, higher values of dehydrogenase activity were recorded in inoculated treatments than in controls as result of a marked increase in microbial activity (Nain et al. 2010). In our study also, higher values of dehydrogenase activity were recorded in T3 (*Providencia* sp.) followed by T1 (*Anabaena oscillarioides*) and T5 (combination of *Providencia* sp. and *Anabaena oscillarioides*) and all the inoculated treatments showed more values than the uninoculated controls.

Seed treatment with soil rhizobacteria such as *Azospirillum*, *Beijerinckia*, *Rhizobium*, *Agrobacterium*, *Bacillus*, *Pseudomonas*, *Mycobacterium*, *Arthrobacter*, *Methylovorus* and *Flavobacterium* strongly stimulates the germination capacity in addition to increasing the growth and crop productivity in plants. Tomato seeds treated with IAA-producing *Pseudomonas putida* showed the highest shoot weight, root weight and shoot length (Gravel et al. 2007). In our investigation, increased root weight, shoot weight and shoot length was observed in treatments in which single and combinations of proteobacterial and cyanobacterial strains were inoculated with wheat seeds. Nezarat and Gholami (2009) reported the highest vigour index when maize seeds were treated with IAA-producing *Azospirillum brasilense* DSM 1690 and *Pseudomas putida* strain R-168, which recorded a vigour index of 975 and 873, respectively. In our study, the highest vigour indices of 7361.2 and 7355.6 were recorded in treatments T3 (WRB4 *Providencia* sp.) and T5 (combination of *Providencia* sp. and *Anabaena oscillarioides*; WRC3 + WRB4), respectively. Strain WRB4 (*Providencia* sp.) exhibited the best performance when used individually or in combination. Burd et al. (2000) reported that PGPR might enhance plant height and productivity by synthesizing phytohormones, increasing the local availability of nutrients and facilitating the uptake of nutrients by plants, which emphasizes the significance of the strains tested in the study.

In our investigation, a positive correlation was recorded between grain weight and plant parameters such as crop biomass, root weight and shoot weight. Interestingly, grain weight also showed a positive correlation with dehydrogenase activity and FDA hydrolysis, revealing the significant role of microbial activity in enhancing crop yields. Crop biomass and microbial activity (dehydrogenase and FDA) were also positively correlated (Figure 2). A positive correlation between crop yield and enzyme activity can be attributed to the improvement in soil chemical and physical properties after soil treatment with effective strains because the enzymatic activities played a role in nutrient cycling, as in our experiment. Similar positive correlations between plant biomass and FDA, and panicle weight and FDA were reported by Nain et al. (2010). It is interesting to note that no published literature on the use of *Providencia* as a PGPR is available because it is primarily investigated as an opportunistic pathogen with limited studies on its potential in bioremediation and as an insecticidal agent.

One of the very important attributes for an effective microbial strain is its competitive ability and saprophytic competence. Khalid et al. (2004) reported that rhizoproteobacteria exhibiting greater competitive ability to survive in soil bring about positive effects on the growth of inoculated plants. In our investigation, the strain of *Providencia* sp. (WRB4) exhibited the ability to utilize 20 different substrates and the strain *Anabaena oscillarioides* (WRC3) utilized 13 different substrates. Interestingly, this strain was able to utilize relatively recalcitrant substrates such as cellulose and melezitose in the dark, which may provide it with a competitive edge over other microflora in the rhizosphere. Cyanobacteria are generally considered to be obligate phototrophs, despite several reports on their photoheterotrophy, and their ability to grow in the dark with simple sugars and produce hydrolytic enzymes (Rippka 1972; Prasanna et al. 2004; 2008, 2009).This strain may also possess such enzymes and heterotrophic abilities and may therefore be a successful inhabitant of the rhizosphere. In comparison, *Alcaligenes* sp. (WRB10) exhibited a narrow spectrum of substrate utilzation, which may be responsible for its lower performance compared with *Providencia* sp. Such strains exhibiting the ability to utilize diverse substrates can survive more competitively under different environmental conditions. A similar increase in plant-growth parameters has been reported from different crops inoculated with rhizosphere-competent *Pseudomonas, Azospirillum* and *Azotobacter* strains (Roesch et al. 2007; Shaukat et al. 2006).

Our results reveal the potential of these IAA-producing strains to enhance not only plant parameters, including crop biomass and grain weight, but also soil microbiological parameters. Our investigation is a first-time report on the plant-growth-promoting role of *Providencia* sp. and *Alcaligenes* in wheat crop and its synergistic interaction with *Anabaena* sp.

Research efforts are needed to elucidate the genes involved and the role of IAA in microorganism–plant interactions, for the effective utilization of these strains as inoculants for wheat crop. Such strains can be employed in integrated nutrient management practices for sustainable agricultural production.

Conclusions

Our study clearly illustrated the potential of selected combinations of proteobacterial genera such as *Providencia* and *Alcaligenes*, and cyanobacteria such as *Anabaena*, as plant-growth-promoting organisms in wheat crop. This also represents a first-time report for the two proteobacterial genera in wheat crop, as also their positive interactions of photoautotrophs with heterotrophs. A definite need exists to utilize such consortia for integrated strategies in sustainable farming of wheat crop.

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