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Growth responses of *Triticum aestivum* after inoculating with *Pseudomonas* and *Stenotrophomonas*

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Three bacterial strains (*Pseudomonas* species, P1; *Pseudomonas mendocina*, P2; and *Stenotrophomonas maltophilia*, S3) were investigated in this study to assess their growth stimulatory effects on *Triticum aestivum* plants. These strains are Gram negative, motile, aerobic rods with catalase and oxidase positive. In general, seed inoculations of these bacteria (mono and mixed cultures) promoted germination, early growth parameters, auxin content, soluble protein content, peroxidase and acid phosphatase activity relative to non-inoculated control seedlings. Mixed cultures inoculations resulted in some decrease in various growth parameters. Almost 15 to 43% decrease in dry weight of plants was observed as compared to non-inoculated control. Bacterial inoculation (*Pseudomonas* sp., P1) resulted in significantly higher acid phosphatase (40%) and peroxidase (39.7%) contents of plant when compared with non-inoculated control. Mixed culture combination showed pronounced synergistic effects relative to respective monocultures.

Key words: *Pseudomonas*, *Triticum aestivum*, auxin, acid phosphatase, peroxidase, *Stenotrophomonas.*

INTRODUCTION

Increase in yield of vegetables, forage and grain crops with inoculation of diazotrophic rhizobacteria, has successfully been demonstrated (Bashan, 1998; Mekonnen et al., 2010). Mechanisms implicated to plant growth stimulation include nitrogen fixation (Pırlak and Kose, 2009), suppression of plant pathogens (Calvo-Bado et al., 2006), mineralization of organic phosphorous or solubilization of inorganic phosphoric compounds, (Dobbelaere et al., 2003), phytohormones production (Ali et al., 2009), root colonization, antibiotics production, siderophore production and enhanced mineral uptake (Dobbelaere et al., 2003).

In plant-microbe interaction, root colonization by beneficial bacteria is the fundamental requirement (Bashan and Holguin, 1994; Qureshi and Sabri, 2011a, b).

Rhizobacteria may sense and respond to plant signals, exchange nutrients with plant cells, suffer damage due to plant defense responses and colonize or even invade root tissues forming symbiotic association (Miller and Wood, 1996). Root lectins of leguminous plants are involved in the recognition and subsequent binding to rhizobia (Diaz et al., 1989). *Azotobacter vinelandii* produces two polymers: the extra cellular polysaccharide alginate and the intracellular polyester poly-ß-hydro butyrate (Castaneda et al., 2000). Alginate is important for cyst formation in *A. vinelandii* as a coating protective polysaccharide material (Nunez et al., 1999). It is suggested that cyst formation and colonization pattern play important roles in regulating nitrogenease activity of plants (Katupitiya et al., 1995).

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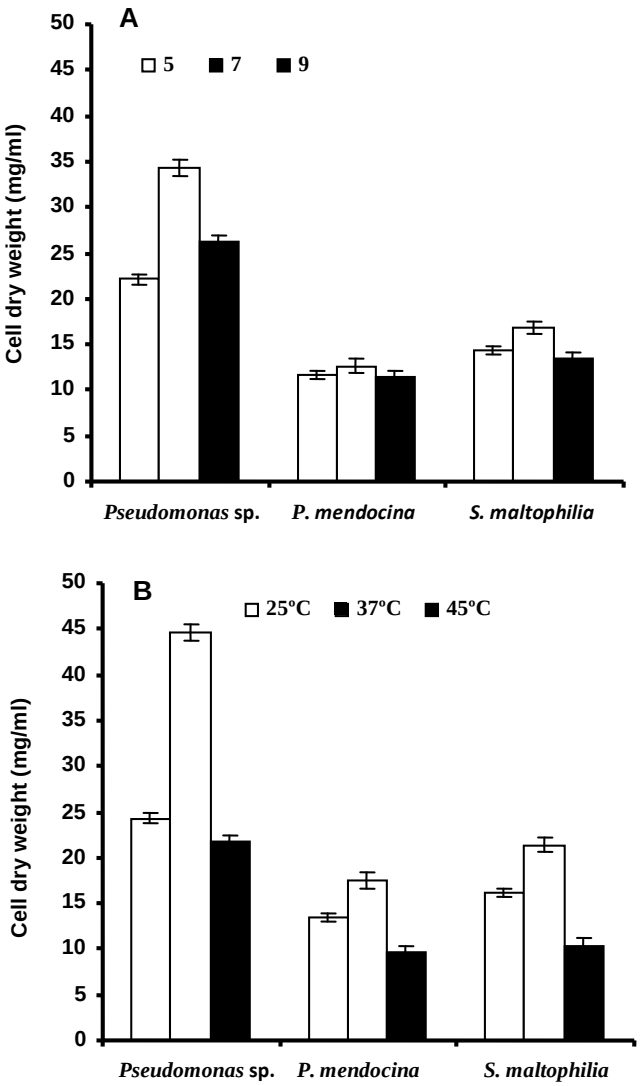

Figure 1. Growth of bacterial isolates at different (A) pH (5, 7 and 9) and (B) temperature (25, 37 and 45°C).

The objective of the present study is to evaluate the impact of mono and mixed culture bacterial strains on the growth and biochemical parameters of *Triticum aestivum* seedlings.

MATERIALS AND METHODS

Bacterial strains and growth conditions

Three bacterial strains (*Pseudomonas* species (P1), *Pseudomonas mendocina* (P2), *Stenotrophomonas maltophilia* (S3)) were used for this work. These bacteria were isolated from rhizosphere (P2 of *Coronopus didyma* and S3 of *Trifolium* species) and histoplane (P1 of *Chenopodium morale*) of different weeds/plants growing in University of the Punjab, Lahore. Three mono and one mix culture of these strains were used for inoculating *T. aestivum* seeds. Bacterial strains were routinely grown on L-agar medium (10.0 g/L tryptone; 5 g/L yeast extract, 5.0 g/L sodium chloride and 12.0 g/L agar for solid media) at 37°C and were stored at 4°C.

Strains characterizations

These strains were characterized morphologically and biochemically following Gerhardt et al. (1994). Bacterial growth was also monitored at various pH (5, 7 and 9) and temperatures (25, 37 and 45°C) in nutrient broth (Figure 1).

Plant-microbe interaction experiments

Fresh cultures of these strains were resuspended in 10 ml of autoclaved distilled water and cell density was adjusted to 10^8 cells/ml with the help of spectrophotometer. Healthy seeds of *T. aestivum* var. Inqlab 91 obtained from NARC Islamabad, Pakistan, were surface sterilized by soaking in 0.1% $HgC1_2$ solution for 5 min with continuous shaking. After that, seeds were washed with sterilized distilled water thrice. Sterilized seeds were then soaked in bacterial suspensions (monoculture and mixed culture suspension) with the help of sterilized forceps for about 15 to 20 min. Ten milliliter of autoclaved distilled water was poured in labeled Petri plate (lined with double layer of Whatman filter paper No. 1 autoclaved and oven dried). With the help of sterilized forceps, seeds (control as well as inoculated) were spread in the respective labeled Petri plate (15 seeds plate) uniformly.

Petri plates were kept in dark at 25 ± 1°C for germination. Germination was recorded daily. After germination, plates were shifted to light (10D lux and 16 h day length) at 25 ± 1°C after adding 10 ml of Hewitt nutrient solution in each plate (Hewitts, 1963). Seedlings were grown for 10 days after shifting to light. Experiment was repeated eight times. Seedlings were removed from the Petri plates and different growth parameters (germination, root length, shoot length, seedling length and dry weight per gram fresh weight) were studied.

Biochemical analysis

For biochemical analysis of both inoculated and control plants, auxin (Mahadevan, 1984), soluble protein (Bhatti et al., 1993; Lowry et al., 1951), peroxidase (David and Murry, 1965) and acid phosphatases content (Iqbal and Rafique, 1987) were studied.

16S rRNA sequencing

To confirm the identity of the strains (P1, P2 and S3), 16S rRNA gene sequencing was undertaken. 16S rRNA gene (1500 bp) was amplified and the amplicon was sequenced using fluorescent dideoxy terminator cycle sequencing chemistry. The extension product was then separated on an ABI PRISM automated DNA sequencer and the data was compared to the MicroSeq® databases (ACCUGENIX™ Newark DE 19702).

Statistical analysis

Data obtained was analyzed statistically following Steel and Torrie (1981). Mean, standard error of the mean, and least significant difference were calculated.

RESULTS

Strains characterizations

All strains were Gram negative, motile, and aerobic rods. They were aerobic in nature. They were unable to hydrolyze

Table 1. Bacterial composition of mono and mixed culture used for inoculating seeds of *T. aestivum*.

Strain	Locality	Occurrence	Source
Pseudomonas sp. (P1)	PU, Lahore	Histoplane	*Chenopodium morale*
P. mendocina (P2)	PU, Lahore	Rhizosphere	*Coronopus didyma*
S. maltophilia (S3)	PU, Lahore	Rhizosphere	*Trifolium* sp.

Table 2. Morphological and biochemical characteristics of strains.

Characteristic	Bacterial strain		
	***Pseudomonas* sp. (P1)**	***P. mendocina* (P2)**	***S. maltophilia* (S3)**
Colony shape	Round	Round	Round
Colony margin	Entire	Entire	Entire
Cell shape	Rod	Rod	Rod
Cell size (µm)	1.5 - 1.8	1.4 - 1.9	0.9 - 1.7
Gram staining	Negative	Negative	Negative
Capsules staining	+	-	+
Motility	+	+	+
Urea	-	-	-
Lactose	+	+	+
D-mannitol	+	+	-
Inositol	-	-	+
D-sorbitol	+	+	-
L-rhamnose	+	+	+
D-sucrose	-	-	+
Oxidase	+	+	-
Catalase	+	+	+
Nitrate reduction	-	-	-
OF test	+	+	+
Starch hydrolysis	+	+	+
Geletin hydrolysis	+	+	+
Plasmid	+	+	+

OF, Oxidation fermentation; -, negative; +, positive.

starch and gelatin. None of the strain was able to reduce nitrate into nitrite (Table 2). They are oxidase and catalase positive. Utilization of different carbohydrates varied in various strains. Optimum growth of these strains was observed at pH 7 and 37°C.

Plant-microbe interaction experiments

Three bacterial strains (P1, P2, and S3) were used for plant microbe interaction studies (Table 1). Besides these three monocultures, one mixed culture of all these strains was used to determine their role in stimulating the germination and early growth of *T. aestivum.*

All bacterial inoculations provoked germination as compared to non-inoculated control seedlings except mix inoculation where some inhibition (4.8%) in germination was recorded (Table 3). Shoot length was markedly increased (36.9%) with the inoculation of strain P1 as compared to the non-inoculated control seedlings. Root lengths of inoculated seedlings were markedly increased except mixed culture which causes slight reduction in the root length as compared to non-inoculated control (Table 3). Seedling lengths of *T. aestivum* were enhanced with the application of bacterial strains as mono and in mix combination as compared to control. Increase in seedling lengths varied from 22.6% in mixed culture combination inoculation to 37% in P1 (monoculture) (Table 4). The number of leaves and the number of roots was not affected by the bacterial inoculations as compared to non-inoculated seedlings. Bacterial inoculations affected differently the weight parameter. All of the inoculations caused decrease in dry weight per gram fresh weight of seedlings as compared to non-inoculated ones (Table 4). Auxin content of *T. aestivum* seedlings was enhanced with bacterial inoculations. Maximum enhancement was achieved with strain S3, where 142% increases in auxin content was recorded in seedlings when compared with

Table 3. Effects of bacterial (mono and mixed culture) inoculations on germination, shoot length and root length of *T. aestivum*.

Strain	Germination (%)	Shoot length (cm)	Root length (cm)
Control	96.8 ± 0.65	6.64 ± 0.38	5.28 ± 0.18
Pseudomonas sp. (P1)	100 ± 0.00	9.09 ± 0.42	7.23 ± 0.38
P. mendocina (P2)	100 ± 0.00	8.14 ± 0.47	6.98 ± 0.46
S. maltophilia (S3)	100 ± 0.00	8.46 ± 0.40	7.40 ± 0.21
Mixed (P1, P2 and S3)	92.2 ± 5.10	8.52 ± 0.70	6.10 ± 0.37
LSD at P = 0.05	2.52	0.224	0.436

LSD: Least significant difference.

Table 4. Effects of bacterial (mono and mixed culture) inoculations on seedling length and dry weight/g fresh weight of *T. aestivum*.

Strain	Seedling length (cm)	Dry weight/g fresh weight (mg/g)
Control	11.92 ± 0.4	112 ± 11.6
Pseudomonas sp (P1)	16.32 ± 0.5	63.5 ± 3.08
P. mendocina (P2)	15.12 ± 0.4	88.6 ± 0.16
S. maltophilia (S3)	15.86 ± 0.6	93.9 ± 7.12
Mixed (P1, P2 and S3)	14.62 ± 0.7	94.3 ± 9.59
LSD at P = 0.05	0.642	5.7

Table 5. Effects of bacterial (mono and mixed cultures) inoculations on auxin content, soluble protein content, peroxidase content and acid phosphatase content of *T. aestivum*.

Strain	Auxin content (µg/g fresh weight)	Soluble protein content (µg/g fresh weight)	Peroxidase content (unit/g fresh weight)	Acid phosphatase content (unit/g fresh weight)
Control	0.52 ± 0.01	524.0 ± 13.2	41.2 ± 3.4	451 ± 12.3
Pseudomonas sp (P1)	0.99 ± 0.01	716.0 ± 14.8	68.4 ± 1.2	754 ± 13
P. mendocina (P2)	1.12 ± 0.02	601.0 ± 21.4	102.4 ± 3.8	534 ± 20
S. maltophilia (S3)	1.26 ± 0.06	748.0 ± 14.8	84.2 ± 2.4	498 ± 13.8
Mixed (P1, P2 and S3)	0.58 ± 0.05	819.0 ± 13.4	98.4 ± 3.6	324 ± 9.7
LSD at P = 0.05	0.132	19.4	2.8	7.1

non-inoculated control seedlings. Mixed culture combination showed synergistic effects and caused a reduction in auxin contents in comparison with mono culture inoculations (Table 5). All the bacterial inoculations caused significant increase in the protein content of the seedlings. Maximum increase was observed with mix cultures (36%) inoculation when compared with the control seedlings (Table 5). In *T. aestivum*, all the bacterial inoculations stimulated the activity of peroxidase significantly relative to non-inoculated seedlings. Maximum increase was observed with P2 inoculation (148%) (Table 5). Acid phosphatase activity of inoculated seedlings increased significantly (40%) with mono culture inoculation, but decreased in the case of mixed culture inoculation (Table 5).

DISCUSSION

Growth promoting bacteria improve plant growth by decomposing mineral material and making availability of nutrients to plants (Dobbelaere et al., 2003), synthesizing and liberating growth hormones (Ali et al., 2009) and reducing the susceptibility to pathogens (Calvo-Bado et al., 2006). Growth stimulating bacteria, especially *Rhizobia*, *Sinorhizobium meliloti* and *Azotobacter* species have been reported to increase the yield and nitrogen content of plants (Khalid et al., 2004).

In this study, three plant growth promoting bacterial strains showed significant stimulation in germination and early growth parameters of *T. aestivum* plants. In general, strain P1 caused maximum enchantment in seedling growth, while in the case of mixed cultures, stimulation in seedling growth was relatively non-significant as compared to non-inoculated control. Reduction in dry weight per gram fresh weight of seedlings occurred in all bacterial inoculations. According to Bashan and Levanony (1990), *Azospirillum* strains could increase the water status of plants which results decrease in dry weight. Decrease in dry weight parameter might be due to unavailability or

uptake of ions and formation of ligands or organic complexes, thus restricting the bioavailability of these ions in the medium (Hughes and Poole, 1989). Alami et al. (2000) studied that inoculation effects of *Rhizobium* strain (isolated from rhizoplane of sunflower roots) on sunflower seeds, which caused increase in shoot and root dry mass under water stress and normal condition. Auxins constitute a class of phytohormones that play important roles in the coordination of plant growth and development. Bacterial inoculations increased auxin content of seedlings markedly relative to control. However, mixed inoculation caused reduction in the auxin content. According to Campbell (1985), the bacterial strains stimulate plant growth by synthesizing and liberating growth hormones. Although, indole-3-acetic acid (IAA) biosynthesis in these bacteria have been shown to occur through different biosynthetic pathways. Thus, the most important mechanism of direct growth promotion may be the production of plant growth regulators (Arshad and Frankenbergrer, 1998). Inoculations (mono and mixed culture treatments) promoted protein content of the seedlings significantly. Auxin and protein are both formed from tryptophan molecules with different arrangements. Hence, the amount of protein content is directly related with auxin content as auxin increase the rate of metabolism, thus increasing the amount of protein content. The phosphatases are diverse class of enzymes. The enzyme activity increases with increasing cell number and cell content in early stages and decreases with maturation. Generally, monoculture inoculations provoked germinations and early growth parameters along with auxin, protein, peroxidase and acid phosphatases contents more effectively than the mixed culture inoculation. One reason may be that bacteria from different sources interfere in the efficiency of one another, whereas in some cases, they showed positive interaction and enhance the activity of one another. From these results, we conclude that bacterial strains which stimulated plant growth promotion individually might portray negative impact when use in combination. Detailed understanding of the inhibitory traits of bacteria would lead to the identification of mechanisms underlying the competition and survival between various groups of bacteria in the rhizosphere.

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