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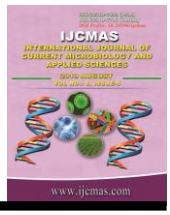

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Screening and Interactive Effects of Soil Microbial Enzymes Activities of Diazotrophic PGPR

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A B S T R A C T

Diverse communities of beneficial plant growth promoting diazotrophs harbour in Rice rhizosphere which mostly plays a significant role in supplementing Biofertilizer, bioprotectant and bio stimulator and improves the crop productivity directly or indirectly. 53 rhizobacterial were isolated from lowland rice rhizosphere of Jorhat district of Assam in non-selective TSA media. 30 pure culture isolates were grown in selective media to and selected five phosphate solubilizers, three *Azospirillum*, one *Azotobacter* and one *Pseudomonas* for screening multiple PGP traits both qualitative and quantitatively. All the selected cultures produced ammonia but Proteolytic activity was limited to few cultures. The test cultures enhanced seed germination ranging from 60-100%, IAA production from 15.8 to 22.6 mg l^{-1} and P-solubilization from 0.3-3.1 percent. The results also indicated direct relationship of drop in pH (6.57 to 4.01) to extent of P-solubilized with days of incubation. A Pot culture experiment was conducted during November, 2016 to evaluate 7 screened cultures of PGP on growth characters of brinjal (*Solanum melongena*) and post-harvest parameters. Among the test PGPR, culture P 20 (*Azospirillium*) recorded significantly highest percent increase in root length (21.6%), plant height (40.6%), root dry weight (120.6%), shoot dry weight (84.82%), total biomass (86.86%), root shoot ratio (19.45%), number of leaves (144.4%) and number of branches (200%) over uninoculated control. On post-harvest parameters, the microbial population was evaluated ranged from 1.7 to 3.5 cfu $10^{6}ml^{-1}$, dehydrogenase activity ranged from 107.2 to 140.4 µg TPF g^{-1}, hydrolysis of Fluorescein diacetate ranged from 64.1 to 80.4 µg FDA g^{-1} hr^{-1} and phosphomonoesterase activity ranged from 75.0 to 94.2 µg p-nitrophenol g^{-1} h^{-1}. *Azospirillium* (P 20) and Phosphate Solubilizing Bacteria (P 49) suggest to be the most efficient biofertilizers, biopesticide and biostimulator properties.

Keywords

Brinjal (*Solanum melongena*), Azospirillum

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Introduction

The rhizosphere is a thin layer of soil which immediately surrounds the plant roots and is the active sites for root activity and microbial proliferation. This zone is rich in nutrients when compared with the bulk soil due to the accumulation of a variety of plant exudates, such as amino acids and sugars, providing a niche area for energy and nutrients source for bacteria. Plant Growth Promoting Rhizobacteria (PGPR) or naturally occurring soil bacteria that aggressively colonize plant roots and accelerate plant growth and

development via direct and indirect mechanism on interaction with the crop by improving productivity and immunity. Plant Growth Promoting Rhizobacteria that fix atmospheric nitrogen by reducing dinitrogen to ammonia or simply N_2 fixers are called 'diazotrophs'. PGPR on inoculation to the crop can directly synthesize phytohormones (IAA) or facilitate the uptake of nutrients and improve soil fertility and indirectly prevent some of the phytopathogens through various mechanisms by producing siderophore, antibiotics, HCN for disease resistance, antimetabolite to avoid predation and competition, β-1, 3, glucanase, chitinase, (Kennedy *et al.,* 2004). Depending on their activity and abundance on the soil environment some of the PGPR in rice rhizosphere are: *Azoarcus, Azospirillum, Bacillus, Burkholderia, Herbaspirillum, Klebsiella* and many other beneficial microbes (Ritu *et al.,* 2017; Hassan *et al.,* 2017). To screen diazotrophic PGP traits and interactive nature of soil microbial enzyme, the present study was undertaken and isolate from rice rhizosphere.

Materials and Methods

The investigation was carried out during 2015-2017, in the department of Soil Science, Assam Agricultural University; Jorhat. 5-10 cm rice rhizospheric soils with the roots were collected randomly from 20 different lowland rice fields under Jorhat district of Assam and isolated the rhizobacteria through serial dilution spread plate technique (Somasegaren and Hoben, 1994) using non selective TSA (Tryptic Soy Agar) media. Diazotrophic PGPR such as P-solubilizer was isolated using Pikovskaya's medium, *Azospirillum* on semi solid NFB (Nitrogen free bromothymol blue) medium, *Azotobacter* on Jensen medium and *Psuedomonas* on the King's B medium. Altogether 10 PGPR were selected as 5 phosphate solubilizers (P 45, P 49, P 50, P 51 and P 53), 3 *Azospirillium* (P 03, P 20, P 28), 1 *Azotobacter* (P 27) and 1 *Pseudomonas* (P 44).

Proteolytic activity

Proteolytic activity was tested using skimmed milk agar medium (Kumar *et al.,* 2005). The selected isolates inoculated into the cut well skim milk agar plates, observed the clear zone around the colonies indicated positive proteolytic activity and recorded the diameter of clear zone in mm.

Phosphate solubilization (Incubation study)

The quantitative estimation of P-solubilization by bacterial isolates was assessed by the vanadomolybdophosphoric yellow color method (Jackson, 1973). The cultures were grown in 50-100 ml aliquots of pikovskaya broth medium containing 0.5% Tricalcium phosphate and incubated upto 30 days in batch at 28±2° C. Centrifuged at 10,000 rpm for 10-15 minutes. 2.5 ml bartons reagent was added 10 ml aliquot and made to final volume 50 ml. The intensity of yellow color was read in a colorimeter at 430 nm wavelength. Samples were drawn at 0, 7, 15 and 30 days interval to assess soluble phosphorus content with days of incubation. The values were calibrated with the standard curve prepared from known concentration of KH_2PO_4. Phosphate solubilizing activity was expressed as percent P released from TCP also the pH of the growth medium was determined at 0, 7, 15 and 30 days.

IAA Production

IAA production by the diazotrophic rhizobacterial isolates was estimated by the method described by Wohler (1997). The rhizobacterial isolates were grown in the Luria-Bertani agar medium (LB) for 72 hrs and centrifuged at 7000 rpm for 5 min.

Removed the supernatant and add 3 ml of phosphate buffer of pH 7.5 along with 1.0% glucose and 1.0% tryptophan into the bacterial pellets and incubated for 24 hrs at 37°C. 2 ml of 5% trichloro acetic acid and 1 ml of 0.5 M $CaCl_2$ were added. Thereafter, the solution was filtered by whatman no.2 filter paper. Each 3 ml filtrates was taken and added 2 ml of Salper's solution (2 ml of 0.5 M $FeCl_3$ and 98 ml of 35% perchloric acid). Incubated for 30 minutes at 25°C in dark until pink color developed. The intensity of pink color developed was measured using UV-VIS spectrophotometer at 535 nm wavelength. The quantities of IAA produced were calculated from the standard curve prepared from the known concentration of IAA and expressed as mg l^{-1}.

Germination properties

The bacteria-plant association released significant amounts of necessary phytostimulator and nutrients from the substrate and help seed germination and establishment. Effect of PGPR on seed germination was performed by semi solid water agar medium. The healthy seeds of wheat, rice and maize were selected, soaked and surface sterilized with 0.1% acidified $HgCl_2$ for 5 mins. After sterilization, the seeds were further washed with series of sterile water to remove the traces of $HgCl_2$. Five seeds of each crop in triplicate were placed onto the semi-solid water agar plates, kept at ambient condition for 30 minutes and thereafter incubated at 30±1°C for 18-48 hrs. The appearance of radical and calculated germination percentage over control using the following formula (Ashrafuzzaman *et al.*, 2009).

$$\text{Germination percentage}(\%) = \frac{\text{Number of seeds germinated}}{\text{Total number of seeds}} \times 100$$

After PGP activities were screened out both qualitative and quantitative, efficient Diazotrophic PGPR were inoculated on Brinjal (*Solanum melongena)*, with variety PPL (long) for determining survivability and endophytic colonizing ability of inoculated isolates with the crop on soil. The experiment was conducted during November, 2016 at Biofertilizer unit, Department of Soil Science, Assam Agricultural University, Jorhat. A total of 9 treatment combinations were replicated thrice were selected as Treatment details: T_1 Absolute control: 6g soil; T_2 Reference control: 6g soil + 30g compost + recommended dose of NPK at 50:50:50; T_3: 6g soil + 30g compost + P 51 (PSB); T_4: 6g soil + 30g compost + P 50 (PSB); T_5: 6g soil + 30g compost + P 49 (PSB); T_6: 6g soil + 30g compost + P 03 (*Azospirillium*); T_7:6g soil + 30g compost + P 20 (*Azospirillium*); T_8:6g soil + 30g compost + P 27 (*Azotobacter*) and T_9: 6g soil + 30g compost + P 44 (*Psuedomonas*). 20 ml inoculum pot^{-1} of active log phase i.e. 10^8 cfu ml^{-1} were inoculated in 2 splits first 10 ml at 7 days after transplanting and the second inoculation of 10 ml was done 15 days after transplanting. The crop was raised for 60 days up to flowering stage and the growth parameter.

Post-harvest parameter in soil

Population dynamics of total bacteria, PSB, *Azospirillium, Azotobacter* and *Pseudomonas* in soil after harvest

The enumeration of total population dynamicswas performed by the method described by Vincent (1970) using spread plate serial dilution technique in appropriate growth medium. The inoculated PSB, *Azospirillium, Azotobacter* and *Psuedomonas* cultures were serially diluted and plated in their respective media such as Pikovskaya's, NFb, Jenson and King's B respectively. The CFU of individual bacterial count was made following the formula as described by Sakthi

et al., (2013).

$$\text{Cfu ml}^{-1} = \frac{\text{No. of colonies} \times \text{reciprocal of dilution used}}{\text{Volume of aliquot taken}} \times 100$$

Dehydrogenase enzyme assay

The quantitative estimation of Dehydrogenase enzyme was performed by the TTC method described by Thalman (1968). 5 g of each soil sample was weighed in 100 ml conical flask and mixed with 5 ml TTC solution, sealed with rubber stopper and incubated for 24 hrs at 30±1°C. The control contained only 5 ml of 100 mM Tris buffer without TTC. Added 40 ml acetone to each flask, shacked thoroughly and incubated at room temperature for 2 hrs in the dark (shaking the flask at intervals). The absorbance of the resultant colour (Red) read at 546 nm wavelength using UV-VIS spectrophotometer. The quantities of Dehydrogenase enzyme in the extract calculated from the standard curve prepared with the known concentration of TPF and expressed the quantity of DHA in µg TPF g^{-1} using the formula:

$$\text{Dehydrogenase activity (µg TPF g}^{-1}) = \frac{\text{TPF (µg/ml)} \times 45}{\text{Dwt} \times 5} \times 100$$

Where, Dwt is dry weight of 1g moist soil, 5 is the moist soil used (g), and 45 is the volume of solution added to the soil sample in the assay

Hydrolysis of Fluorescein Diacetate

The quantitative estimation of hydrolysis of Fluorescein Diacetate enzyme was performed by the method described by Schnurer and Rosswall (1982). 1 g of each of soil sample was suspended in sterile culture tube and added 7.5 ml of potassium phosphate buffer (60 mM, pH 7.6) and allowed to equilibrate at 25°C on shaker. After shaking, 0.1 ml FDA solution (1000 µg/ml) was added, again shaked in the shaker and further incubated at 25°C for 30 minutes. For blank, 0.1 ml acetone was added instead of 0.1 ml FDA and incubated for 30 min. After incubation, 7.5 ml of extractant (chloroform: methanol; 2:1) was added to both the sample and blank to stop the reaction. The lid was replaced and mixed the content thoroughly in vortex for 10 sec. Thereafter centrifuged the mixture at low speed (RCF=300× g for 2 min) and transferred 1.2 ml upper phase to 1.5 centrifuge tube and again centrifuged at (RCP=16,500 × G for 5 min) to remove suspended particles. One ml of supernatant was taken in the cuvette and measured OD at 490 nm on spectrophotometer against blank. The quantity of Fluorescein was calculated from the standard curve prepared with the known concentration of fluorescein and expressed in µg FDA g^{-1} hr^{-1} as follows:

$$\text{Fluorescein Diacetate, (µg FDA g}^{-1}\text{ hr}^{-1}) = \frac{\text{FDA (µg/ml)} \times 15}{\text{Dwt} \times 1}$$

Where, dwt is dry weight of 1g moist soil, 1 is the moist soil used (g), and 15 is the volume of the solution added to the soil sample in the assay.

Phosphomonoesterase

The quantitative estimation of Phosphomonoesterase enzyme was performed by the method described by Tabatabai and Bremner (1969). 1 g of each of soil sample was weighed in 50 ml conical flask and added 4 ml MUB along with 0.25 ml toluene and 1 ml of 115 mM p-nitrophenyl phosphate solution. The content was swirled and incubated at 37°C for 1 hr. After incubation, 1 ml of 0.5 M $CaCl_2$ and 4 ml of 0.5 M NaOH was added and mixed thoroughly. After filtration, the yellow color filtrate determined the absorbance at 400 nm wavelength. The quantities of Phosphomonoesterase enzyme

calculated from the standard curve prepared with the known concentration of p-nitrophenol and expressed in µg p-nitrophenol g^{-1} h^{-1}using the formula:

$$\text{Phosphomonoesterase enzyme } (\mu g \text{ p-nitrophenol } g^{-1} h^{-1}) = \frac{C \times V}{Dwt \times SW \times t}$$

Where, C is the measured concentration of p-Nitrophenol (µg/ml filtrate), dwt is the dry weight of 1 g moist soil, V is the total volume of the soil suspension in milliliters, SW is the weight of soil sample used (1g) and t is the incubation time in hours. The data obtained were analysed through completely randomized design and two factorial completely randomized design. The significance of variance was determined by calculating the respective F-value and significance of specific means differences was determined by calculating the critical differences (C.D) at 5% level of significance.

Results and Discussion

Mineral P-solubilization, ammonia production, Proteolytic activities and IAA production were examined from 10 Diazotrophic PGPR (P-Solubilizers, *Psuedomonas*, *Azotobacter* and *Azospirillium*) Qualitatively all the diazotrophic isolates were capable to produces ammonia (Table 1). 7 out of 10 test PGPR cultures were proteolytic producer with varied intensity where culture P 20 recorded highest proteolytic activity followed by P 03 (Table 1).

The diazotrophic proteolytic activator can act as exotoxin against phytopathogen by the action of protease enzyme**.** The selected Diazotrophic PGP cultures produces IAA ranged from 15.88 to 22.66 mg l^{-1}. The culture P 03 produced the highest IAA (22.66 mgl^{-1}) which was significant followed by culture P 20 (22.57 mgl^{-1}) which was at par with culture P 03. The lowest IAA production was recorded with P 45 (15.88 mgl^{-1}) (Table 1). Thus, culture P 20 and P 03 was significant producer of IAA (Shrivastava *et al.,* 2014; Gururani *et al.,* 2014). The selected diazotrophic PGPR showed 60 to 100 percent seed germination ranged over control on wheat, rice, and maize seeds using water agar medium (Table 2). 100 per cent germination of wheat, rice and maize seeds recorded by culture P 20 (*Azospirillium*) and P 44 (*Azotobacter*). Result revealed that due to production of phytohormone (IAA) and mineral nutrient as shown in diazotrophic PGPR exhibit the highest seed germination.

Analysis of variance (Table 2), the diazotrophic PGP cultures, P 49 recorded significantly the highest P-solubilization at 7 days (2.383%), 15 days (2.866%) and even at 30 days (3.173 %). On the contrary, culture P 28 recorded lowest per cent solubilization at 7 and 15 days. The results (Table 2) showed variation among the test cultures in solubilizing phosphate with days of incubation. The interaction effect of pH with days of incubation was found significant as days of incubation increase with corresponding decrease in pH. Table 2 showed that mean decrease in pH ranged from 6.57 (at 0 days) to 4.01 (at 15 days) and thereafter, the pH raised to 5.24 at 30 days. The results clearly showed the relationship of P-solubilization with decrease in pH, which indicated that, a role of organic acid secretion by microbes to solubilizes insoluble phosphate of TCP with days of incubation.

On pot culture evaluation (Table 3 and 4), culture P20 showed superiority on enhancing biological growth character in brinjal which was evident from the data of root length, shoot length, plant height, root dry weight, shoot dry weight etc all increased due to inoculation treatment.

Table.1 PGP activities produced by diazotrophic PGPR cultures

Sl. No.	Isolates	Germination Percentage (%)			Ammonia Production	Proteolytic activity Diameter of clear halo zone (mm)	IAA (mgl^{-1})
		Wheat	Maize	Rice			
1	P 03 (*Azospirillium*)	100	80	100	+	21	22.66
2	P 20 (*Azospirillium*)	100	100	100	+	22	22.57
3	P 27 (*Azotobacter*)	80	80	100	+	20	18.68
4	P 28 (*Azospirillium*)	60	100	80	+	-	19.76
5	P 44 (*Psuedomonas*)	100	100	100	+	17	21.34
6	P 45 (PSB)	86.66	60	60	+	-	15.88
7	P 49 (PSB)	80	80	100	+	20	20.45
8	P 50 (PSB)	80	80	80	+	18	19.06
9	P 51 (PSB)	60	100	100	+	-	18.83
10	P 53 (PSB)	60	60	80	+	16	17.65
11	Control	53.33	60	60		-	4.13
						S.ED (±)	0.375
						$C.D_{0.05}$	0.777

Table.2 Effect of diazotrophic PGP cultures on P-solubilization (%) as affected by days of incubation and Change in pH corresponding to its days of incubation

Isolates	P-solubilization (%) with days of incubation Change in pH with days of incubation				Mean	Change in pH with days of incubation				Mean
	0 Days	7 Days	15 Days	30 Days		0 Days	7 Days	15 Days	30 Days	
Control	0.301	0.306	0.303	0.340	0.312	6.647	6.617	6.641	6.665	6.642
P 03 (Azospirillium)	0.377	2.040	2.475	2.718	1.903	6.650	4.535	3.968	5.091	5.061
P 20 (Azospirillium)	0.365	2.180	2.654	2.996	2.049	6.685	4.544	3.544	4.932	4.926
P 27 (Azotobacter)	0.372	1.548	2.148	2.401	1.617	6.692	4.538	4.004	5.194	5.107
P 28 (Azospirillium)	0.384	0.931	1.734	2.634	1.421	6.654	4.945	4.445	5.516	5.390
P 44 (Psuedomonas)	0.349	2.095	2.434	2.672	1.888	6.490	4.394	3.827	5.341	5.013
P 45 (PSB)	0.350	2.034	2.436	2.796	1.904	6.528	4.471	3.637	5.232	4.967
P 49 (PSB)	0.383	2.383	2.866	3.173	2.201	6.658	4.382	3.315	4.541	4.724
P 50 (PSB)	0.375	2.079	2.376	2.932	1.940	6.437	4.464	3.564	4.995	4.865
P 51 (PSB)	0.373	1.772	2.338	2.893	1.844	6.452	4.488	3.588	4.992	4.880
P 53 (PSB)	0.347	1.834	2.600	2.873	1.914	6.433	4.485	3.585	5.136	4.910
Mean	0.361	1.746	2.215	2.584		6.575	4.715	4.011	5.240	
		S.ed (±)		$CD_{0.05}$				S.ed (±)	$CD_{0.05}$	
Cultures		0.012		0.025		pH		0.031	0.061	
Days		0.004		0.009		Days		0.011	0.022	
Interaction (Cultures×days)		0.051		0.101		Interaction (pH × days)		0.124	0.247	

Table.3 Effect of diazotrophic bacterial inoculation on Root Shoot ratio of Brinjal

Treatments	Root dry weight (g)	% increase over control	Shoot dry weight (g)	% increase over control	Total biomass (g)	% increase over control	Root shoot ratio	% increase over control
T_1 = Uninoculated control	0.305	-	5.037	-	5.342	-	0.060	0.000
T_2 = Standart (50:50:50)	0.524	71.991	8.167	62.129	8.691	62.692	0.064	6.215
T_3 = P 51 (PSB)	0.552	81.182	8.562	69.978	9.114	70.616	0.064	6.642
T_4 = P 50 (PSB)	0.523	71.772	8.158	61.951	8.681	62.511	0.064	6.135
T_5 = P 49 (PSB)	0.646	112.144	9.214	82.908	9.860	84.575	0.070	16.033
T_6 = P 03 (*Azospirillium*)	0.587	92.779	8.774	74.186	9.362	75.246	0.067	10.725
T_7 = P 20 (*Azospirillium*)	0.672	120.678	9.310	84.820	9.982	86.865	0.072	19.456
T_8 = P 27 (*Azotobacter*)	0.519	70.460	8.438	67.516	8.958	67.684	0.062	2.034
T_9 = P 44 (*Pseudomonas*)	0.588	92.998	8.752	73.743	9.340	74.841	0.067	11.064
S.Ed. (±)	0.049		0.140		0.150		0.005	
$C.D_{0.05}$	0.104		0.295		0.316		0.012	

Table.4 Effect of diazotrophic bacterial inoculation on root length (cm), shoot length (cm), plant height (cm) number of leaves and branches of brinjal

Treatments	Root length (cm)	Percent increase over control	Shoot length (cm)	Percent increase over control	Plant height (cm)	Percent increase over control	Number of branches	% increase over control	Number of leaves	% increase over control
T_1 = Uninoculated control	6.93	0	25.01	0	31.95	0	1.333	-	6	-
T_2 = Standart (50:50:50)	8.00	15.38	34.37	37.39	42.37	32.62	3.000	125	10.667	77.778
T_3 = P 51 (PSB)	8.30	19.71	35.73	42.86	44.03	37.83	3.333	150	11.000	83.333
T_4 = P 50 (PSB)	7.80	12.50	34.50	37.93	42.30	32.41	3.000	125	10.333	72.222
T_5 = P 49 (PSB)	8.37	20.67	36.53	46.06	44.90	40.55	3.667	175	14.333	138.889
T_6 = P 03 (*Azospirillium*)	8.27	19.23	35.17	40.59	43.43	35.96	3.333	150	13.667	127.778
T_7 = P 20 (*Azospirillium*)	8.43	21.63	36.50	45.92	44.93	40.65	4.000	200	14.667	144.444
T_8 = P 27 (*Azotobacter*)	7.97	14.90	33.39	33.48	41.35	29.44	2.667	100	10.000	66.667
T_9 = P 44 (*Pseudomonas*)	8.33	20.19	35.34	41.28	43.67	36.71	3.667	175	13.000	116.667
S.Ed. (±)	0.124		0.299		0.269		0.384		0.351	
$C.D_{0.05}$	0.262		0.628		0.565		0.966		0.738	

Table.5 Population dynamics of total bacteria, PSB, *Azospirillium*, *Azotobacter* and *Pseudomonas* after harvest, DHA activity, Hydrolysis of fluorescein diacetate and Phosphomoesterase activity by diazotrophic PGPR

Treatment detailed	CFU (10^6ml^{-1})	DHA Activity (µg TPF g^{-1})	Hydrolysis of fluoresin diacetate (µg FDA g^{-1} hr^{-1})	Phosphomoesterase activity (µg p-nitrophenol g^{-1} h$^{-1)}$
T_1 = Uninoculated control	2.5	70.76	32.509	29.399
T_2 = Standard (50:50:50)	3.5	102.8	62.019	72.237
T_3 =P 51 (PSB)	2.1	109.24	64.193	86.738
T_4 =P 50 (PSB)	1.7	107.22	69.969	83.221
T_5 =P 49 (PSB)	3.1	125.83	75.125	94.231
T_6 = P 03 (*Azospirillium*)	2.8	120.84	79.265	77.090
T_7 =P 20 (*Azospirillium*)	3.3	140.40	80.495	91.152
T_8 =P 27 (*Azotobacter*)	3.0	116.38	73.561	75.040
T_9 = P 44 (*Pseudomonas*)	2.0	112.50	64.249	86.595
S.Ed.(±)	-	0.355	0.277	0.195
$CD_{0.05}$	-	0.746	0.582	0.410

For instances, P 20 recorded highest per cent increased in root dry weight (120.6 %), shoot dry weight (84.82 %), total biomass (86.86 %), root shoot ratio (19.45 %), root length (21.6 %), plant height (40.6 %), no. of leaves (144.4%) and no. of branches (200%) over uninoculated control. Therefore, culture P 20 was regarded as superior PGP cultures in enhancing growth characters of brinjal followed by culture P49 (PSB).

The microbial colony was enumerated by using their respective media. Table 5 showed that overall bacterial population was recorded highest in standard treatment with 3.5 cfu 10^6ml^{-1}. However, the highest established population of *Azospirillium* was recorded in P 20 (3.3 cfu 10^6ml^{-1}) followed by P 49 (3.1 cfu 10^6ml^{-1}) and P 27 (3.0 cfu 10^6ml^{-1}). Large bacterial population was detected in standard treatment indicating colonization by indigenous bacteria. These results the isolates were able to survive and colonize in the rhizospheric soil despite of competition by native microflora.

The dehydrogenase activity (DHA) is purely a microbiological origin, and represents the oxidoreduction process in the respiration process which is also a good indicator of soil microbiological activity (Singh and Singh, 2005). DHA was recorded significantly highest in all the inoculated treatment over control and ranged from 107.2 to 140.40 µg TPF g^{-1} where P 20 recorded highest DHA and lowest in P 50 (Table 5). Thus, the diazotrophic bacterial cultures were capable in mineralizing fresh organic matter and humus relating to soil fertility. However, the value of DHA varied among the treatments because of diverse active microbial population abundance in the soil, effect of the substrate and soil condition.

Fluorescein diacetate is a membrane-bound enzymes such as esterases, proteases, and lipases, resulting in the release of fluorescein by soil microorganism and inhibited the deleterious microbes (Schnurer and Rosswall, 1982). FDA was recorded significantly highest in inoculated treatments over control ranging from 64.19 to 80.5 µg FDA g^{-1} hr^{-1} where culture P 20 recorded the highest FDA and the lowest FDA with P 50 (Table 5). The variation of FDA among the treatments was recorded because of diverse activity and density of the introduced PGPR associated with various non-specific enzyme such as esterases, proteases, and lipases. The above data could be reported that the selected isolates were capable of suppressing the growth of pathogen indirectly through proteases activity. Thus, the application of PGPR is a potential alternative approach in disease management.

P 49 (PSB) (Table 5) recorded significantly highest phosphomonoesterase activity (94.23 µg p-nitrophenol $g^{-1}h^{-1}$) and lowest Phosphomonoesterase activity in P 27 (75.04 µg p-nitrophenol g^{-1} h^{-1}) which clearly resulted that P-solubilizer efficiently mineralized P by the action of phosphomonoesterase enzyme in soil organic matter and therefore promotes the dispersal and activity of phosphorus (Nannipieri *et al.,* 2011).

The recent investigation, gives a clear picture about the role of diazotrophic bacteria on inoculating to Brinjal crop enhanced growth and development of the crop through direct and indirect PGP activities such as production of IAA, Ammonia, Mineral P-solubilization and proteolytic activity. Diazotrophic PGP were also capable to colonize and survived with various microbial enzyme activities such as Dehydrogenase, FDA and Phosphomonoestease. Thus, it can be concluded that the Diazotropic PGPR are the promising alternative biopesticides, biostimulator and biofertilizer.

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