

Influence of Fertilizer Level on Establishment of LacZ Tagged Mutants of *Pseudomonas striata* in Soybean Rhizosphere

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Abstract: The fates of *Pseudomonas striata* (P-27) and its mutant were compared in the rhizosphere of soybean at different fertilizer level. Three lacZ tagged mutant strains designated as superior, inferior and homologous to parent with respect to P-solubilization (T-80, T-128, T-125) and IAA production (T-49, T-57, T-87) were selected for establishment studies. The comparison of mutants for their ability to survive and establish in soybean rhizosphere revealed, a linear decline of the population with plant growth period. Individual pot experiments were conducted with superior strains to study the influence of level of fertilizer on phosphobacterial population in rhizosphere and crop growth. It is concluded that inoculation of *P. striata* strain increases the population of phosphobacteria and growth parameters of soybean irrespective of the level of fertilizer. The application of fertilizer beyond 50% of the recommended dose was found to be relatively unresponsive.

Key words: Missing

INTRODUCTION

Current developments in crop sustainability involve a rational exploitation of soil microbial activities, which for potential environmental benefits, leading to reduction in the use of agricultural chemicals. The prospects of manipulating crop rhizosphere microbial populations by inoculation of beneficial bacteria to increase plant growth have shown considerable promise in laboratory but responses have been variable in natural conditions [1]. The performance of microbial inoculants depends largely on survival, successful establishment and root colonization under *in situ* conditions [2, 3]. Colonization of roots by inoculated microorganisms is very complex and crucial process, which is not fully understood. Although several microbial species have been released in large quantities in the environment, very little is known about the ecology of phosphobacteria. To develop an insight of the process, the introduced strains need to be monitored to assess their fate and in developing improved application strategies [4].

In soil, fate of inoculated microorganisms is also dictated by factors including agricultural practices. Fertilizers are used rampantly to increase the crop yield by restoring and maintaining soil fertility irrespective of use of microbial inoculants. Fertilizers being direct source of inorganic nutrients could therefore play a major role, affecting microbial population in rhizosphere. In addition, it would be useful to explore a possibility of cutting down the dose of fertilizers by incorporating phosphobacterial inoculants. Thus, the aim of present investigation was to monitor establishment of lacZ tagged strains of *P. striata* in soybean rhizosphere and to study survival of inoculated strains and their effect on plant growth parameters at different levels of fertilizer application.

MATERIALS AND METHODS

Bacterial Strains and Plasmid: *Pseudomonas striata* (P-27) and its lacZ tagged mutants were selected for their ability to solubilize inorganic phosphorus in culture

medium from a previous study [5]. *P. striata* (P-27) and mutants were routinely maintained on Pikovskaya (PV) and Luria Bertani (LB) medium respectively [6, 7]. Antibiotics were added to growth media of lacZ tagged mutants: Kanamycin (Kan: 50 µgG¹), Nalidixic acid (Nal: 50 µgG¹). lacZ tagged mutants of phosphobacteria from soybean rhizosphere were selected on LB agar with antibiotics, IPTG and X-gal. Long term stock strains were maintained in 25% glycerol.

Strain Selection and Plant Inoculation: Seeds of soybean cultivar Pusa-22 were surface sterilized in acidified 0.1% mercuric chloride solution with a drop of Tween-20 for 5 min and washed 4-5 times with sterile distilled water. Seeds were inoculated with parent and mutant strains of *P. striata*. Six strains adjudged superior, inferior and homologous to parent with respect to P-solubilization (T-80, T-128, T-125) and IAA production (T-49, T-57, T-87) were used for seed inoculation. The P solubilizing strains were poor IAA producers and *vice versa*. The population of all the strains was adjusted to 10⁸ colony forming units per milliliter (cfu mlG¹) prior to seed inoculation.

Rhizosphere Establishment Trials: Studies on establishment of inoculated strains were performed in pot experiment under natural conditions. The experimental unsterilised soil from Todapur block of IARI farm, New Delhi was sun dried and sieved through a 2 mm sieve. Soil was treated with a basal dose of finely ground and well decomposed farm yard manure (FYM @10 tonne haG¹) along with recommended fertilizer dose (N, P, K @ 20:60:40 kg haG¹). The soil was filled in earthen pots (10⁰ diam) containing one inch of gravel. Plants were watered daily with tap water as required in maintaining soil at field capacity. Plant protection measures were taken up whenever required. The rhizospheric population of parent and mutant strains was monitored at different stages of plant growth. Inoculant population was enumerated by direct plating of rhizospheric soil samples on screening medium. For each inoculation treatment, triplicate samples were collected at 10, 45, 60, 90 and 120 days of plant growth. At each sampling time root system were shaken vigorously to dislodge loosely adhering soil. 1 g of soil adhering to root systems was serially diluted and 100 µl of appropriate dilutions were spread over surface of PV medium and screening medium and incubated for 2-7 days prior to colony enumerations. Colony forming units were expressed as cfu gG¹ of soil. PV medium was used for enumeration of native phosphobacteria in control.

Effect of Fertilizer Amendment on Establishment of Phosphobacteria: The effect of fertilizer use in soil on establishment of phosphobacteria was studied at three levels of N, P and K. Soil used for the pot experiment was amended with 0, 50 and 100% of the recommended dose of FYM, N, P and K. The parent (P-27) and superior strains with respect to P-solubilization (T-80) and IAA production (T-49) were selected for seed inoculation [5]. The data on colonization of phosphobacteria, dry matter yield, grain yield, N and P-uptake of crop were recorded.

Assessment of Plant Yield Parameters: On harvesting the crop, the shoots were cut 1 cm from the base and pods removed from the plants. The plant parts were dried in oven at 70°C for 48 hrs. The dry weight was measured until a constant weight was recorded. Dried shoot and grain samples was ground using mechanical grinder with 0.5 mm sieve. Total nitrogen of plant samples was determined by standard Kjeldahl method using N-autoanalyzer (Technicon, Dublin) [8]. The ammonium concentration was automatically recorded as a peak by measuring optical density at 660 nm (OD₆₆₀). The nitrogen (N) concentration was worked out using a calibration curve and expressed as N (%) on dry weight basis. Total phosphorus (P) content in shoot and grain was determined by vanadomolybdo-phosphoric yellow complex method as described (Jackson, 1973). The optical density was measured at 470 nm (OD₄₇₀) using added reagent blank as reference. The phosphorus content of the plant sample was calculated using a standard curve.

Statistical Analysis Statistical analyses were performed on triplicate samples. Comparison was made by analysis of variance and standard errors of difference calculated [9]. For bacterial densities, evaluation data were log transformed prior to statistical analysis.

RESULTS

The parent and mutant strains of *P. striata* colonized soybean rhizosphere over the period of plant growth. The differences in the number of mutant strains that colonized soybean rhizosphere were significant when compared with un-inoculated control (Table 1). However, the differences among strains were marginal. Mutants showed linear decline in their population with respect to plant growth period. The parent strain (P-27) showed maximum rate of population decline (-1.10) whereas the mutant strain (T-125) was minimum (-1.24) in this regard (Fig. 1). The screening medium could select the

Table 1: Colonization of soybean rhizosphere by phosphobacterial strain in response to fertilizer application

<i>P. striata</i> strain (S)	Phosphobacteria (log cfu g ⁻¹ soil)			Mean
	Fertilizer level (F)			
	0	50	100	
Parent (P-27)	5.85	6.49	6.60	6.32
Mutant (T-80)	5.98	6.51	6.69	6.35
Mutant (T-49)	5.89	6.50	6.62	6.34
Control	3.83	4.16	4.46	4.15
Mean	5.39	5.92	6.07	
	S	F	S x F	
S. Em ±	0.01	0.01	0.02	
C.D. at 5%	0.04	0.05	0.08	

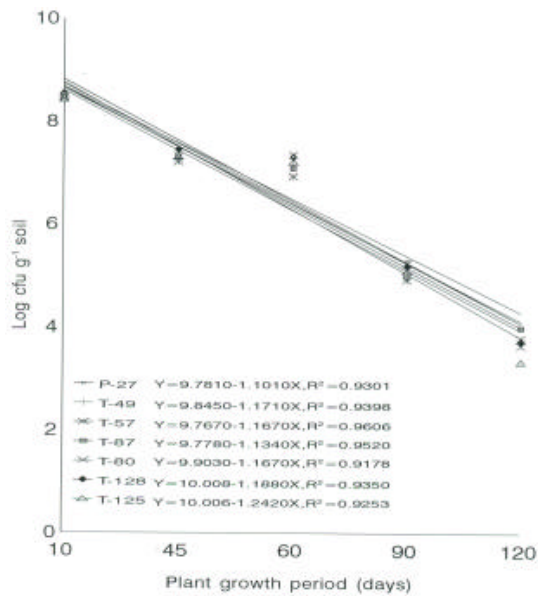


Fig. 1: Colonization of soybean rhizosphere by inoculated strains of *P. striata*

population of lacZ tagged mutants irrespective of their plant growth promoting trait.

Application of fertilizer influenced phosphobacterial population in rhizosphere of soybean (Table 1). The increase in phosphobacterial population was significant up to half the recommended rate of fertilizer application. Any further increase in fertilizer application did not yield a proportionate response. Increase in fertilizer level application from 0-50 and 50-100% increased the number of phosphobacteria in the rhizosphere by 9.83 and 2.53%, respectively.

Plant growth response to inoculation improved with the application of fertilizer. The maximum response in plant growth parameters were recorded with inoculation of mutant T-80 which was superior in P solubilization among the tested strains. Seed inoculation with the strain T-80 resulted in 39.00, 140.73 and 61.23 % increase in dry matter yield, P and N-uptake of shoot respectively over the uninoculated control at recommended level of P application (Table 2). Increases in yield, P and N uptake of grain in this regard were 28.75, 40 and 39.13 % at half the recommended level whereas 15.75, 19.20 and 25.86 % at recommended level of fertilizer (Table 3). The maximum response in plant growth parameters of shoot and grain were recorded with mutant T-80 which was most superior in P solubilization among the inoculated strains.

DISCUSSION

The parent and mutant strains of *P. striata* were comparable as their population registered a decline over the period of plant growth (Table 1 and Fig. 1). A decline in the population of *P. fluorescens* R2fR and its mutant derivative R1WE8 tagged with lacZ marker was reported in wheat rhizosphere [10]. Microorganisms introduced into the rhizosphere frequently exhibit a decline in

Table 2: Dry matter yield, P and N-uptake of shoot in response to fertilizer application and colonization of phosphobacterial strain

<i>P. striata</i> strain (S)	Shoot											
	Dry Wt. (g pot ⁻¹)				P uptake (mg pot ⁻¹)				N uptake (mg pot ⁻¹)			
	Fertilizer level (F)				Fertilizer level (F)				Fertilizer level (F)			
	0	50	100	Mean	0	50	100	Mean	0	50	100	Mean
Parent (P-27)	9.77	10.07	11.07	10.30	26.48	35.93	43.13	35.18	254.40	284.21	315.99	288.20
Mutant (T-80)	10.65	11.47	12.15	11.43	29.72	40.26	48.34	39.44	260.34	290.01	334.16	294.84
Mutant (T-49)	9.93	10.33	11.04	10.43	26.99	37.99	46.05	37.01	265.93	286.69	329.56	291.06
Control	8.74	8.99	9.87	9.20	20.08	24.80	29.49	24.49	207.25	220.99	231.57	216.94
Mean	9.78	10.21	11.03		25.82	34.74	41.75		244.73	270.48	305.32	
	S	F	S x F		S	F	S x F		S	F	S x F	
S. Em±	0.04	0.03	0.06		0.31	0.27	0.54		0.59	0.51	1.01	
C.D. at 5%	0.17	0.20	0.21		1.41	1.65	1.88		2.63	3.08	3.51	

Table 3: Yield, P and N-uptake of grain in response to fertilizer application and colonization of phosphobacterial strain in soybean rhizosphere

<i>P. striata</i> strain (S)	Grain											
	Dry Wt. (g pot ⁻¹)				P uptake (mg pot ⁻¹)				N uptake (mg pot ⁻¹)			
	Fertilizer level (F)				Fertilizer level (F)				Fertilizer level (F)			
	0	50	100	Mean	0	50	100	Mean	0	50	100	Mean
Parent (P-27)	8.67	11.93	13.83	11.47	44.63	64.07	78.54	62.41	425.2	595.5	768.6	596.47
Mutant (T-80)	10.09	12.92	15.36	12.79	47.41	67.15	81.93	65.50	468.9	639.4	843.8	656.71
Mutant (T-49)	9.12	11.50	14.12	11.58	45.24	64.83	79.14	63.07	456.3	613.5	781.4	617.1
Control	7.06	8.62	9.08	8.25	35.52	41.61	43.77	39.32	257.5	413.8	453.5	374.97
Mean	8.73	11.24	13.01		42.45	59.43	70.84		406.5	565.5	711.84	
	S	F	S x F	S	F	S x F	S	F	S x F	S	F	S x F
S. Em±	0.33	0.28	0.56	0.38	0.33	0.66	0.59	0.51	1.02			
C.D. at 5%	1.46	1.71	1.95	1.70	1.99	2.27	2.65	3.10	3.53			

population to or below detection limits within a period of months. Although exceptions exist, introduced microorganisms do not maintain the initial artificially high population densities. Slow decline in bacterial density over a period may indicate a change in root exudates or change in cumulative effects of competition in the rhizosphere [11, 12]. The setup of the experiment performed in this study was restricted to small scale to circumvent complication caused by heterogeneities commonly occurring in large field experiments. The pot culture experiments carried out represent a transition between controlled laboratory experiments under constant environment and open fields with ever fluctuating soil and environmental conditions.

The constitutive expression of heterologous genes may represent a metabolic burden for the bacteria host in soil, resulting in an impairment of its fitness [13]. However, similar survival pattern of parent and mutant strains as judged by cfu counts did not reveal effect of genetic insert during the growth period of soybean. The rate of decline of mutants, superior or inferior in plant growth promoting traits was similar to parent (Fig. 1).

Fertilizers being inorganic sources of nutrients can meet the crop requirement efficiently. However, application of fertilizers imposes an economic and environmental stress. Studies were therefore, conducted with different levels of fertilizers application to explore a possibility of cutting down the level of fertilizers by incorporating microbial systems for increasing availability of phosphorus. Significant increase in rhizospheric population of phosphobacteria and other plant growth parameters viz. yield, P and N-uptake of shoot and grain was observed with fertilizer application however, the maximum increase (%) in most parameters was recorded at 50% application of recommended fertilizer

level (Table 1, 2 and 3). The stimulating effect of fertilizer may be directly due to the increased supply of available nutrients not only to crops but microbial population and indirectly through changing the growth rate and metabolic activities of plant [14]. Failure in proportional response to doses higher than 50% may be explained by PUF (utilization of phosphorus from fertilizer source) of soybean as described [15]. These workers reported that the PUF was greater with the smaller rates of P application in the case of soybean. The decrease observed in PUF was drastic beyond 40 kg ha⁻¹. The possible reason for the maximum PUF at lower rates of application and decreasing trend observed with graded rates of applied P could be that the plant have a greater demand of P at lower rates of application as experimental soil was deficient in available P. Failure in proportional response may also be due to higher fixation or loss of nutrients at higher doses of fertilizer application. The soluble phosphate fertilizer compounds once applied are immobilized at high rate [16]. The experimental data showed that inoculation of *P. striata* resulted in significant increase in dry matter and yield parameters of soybean. Increases in crop yield parameters were recorded in field experiments with soybean under rain fed condition under different agroclimatic location [17]. The favorable effect of inoculation of *P. striata* on colonization of phosphobacteria, plant growth and nutrient uptake was mainly due to improved phosphate nutrition and production of growth promoting substances [18]. Majority of plant growth experiments on P-solubilization in soil have been conducted under green house conditions. In the green house rooting volume are usually restricted. The contribution of soil-borne P to plant nutrition is reduced and response to P solubilized by phosphobacteria will be greater. Under

these condition increases in P uptake and plant growth parameters in various crops inoculated with phosphobacteria have been reported [19, 20]. Increase in various growth parameters of shoot and grain with recommended level of fertilizer application in control treatments could be achieved with only 50% fertilizer dose when inoculated with superior strain of *P. striata* (Table 1, 2 and 3). This is indicative of the fact that by incorporating microbial systems with improved PGPR activities hold promise of increasing crop yield while sustaining the environment by cutting down the use of inorganic fertilizer.

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