



## Impact of soil temperature, pH and carbon dioxide on the population and efficiency of fluorescent pseudomonad in the rhizosphere soil of *Pokkali* rice

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ARTICLE INFO	ABSTRACT
<p>Received : 18 December 2021                      Revised : 27 March 2022                      Accepted : 16 July 2022</p> <p>Available online: 08.01.2023</p> <p><b>Key Words:</b>                      Fluorescent pseudomonads  <i>Pokkali</i> rice                      Soil parameters                      Soil temperature</p>	<p>The present study was aimed at the evaluation of soil temperature, pH and carbon dioxide evolution on the number and efficiency of fluorescent pseudomonads around the root system of <i>Pokkali</i> rice at Vytilla in Ernakulam district of Kerala. Two plots (40 m<sup>2</sup>) comprising control (without application of <i>Pseudomonas fluorescens</i>) and <i>P. fluorescens</i> treated plants were used for the field experiment. The isolates of fluorescent <i>Pseudomonads</i> or <i>Pseudomonas fluorescence</i> were counted and their efficiency was assessed for IAA, ammonia, HCN and siderophore production. Simultaneously, soil temperature, pH, and carbon dioxide evolution were also recorded. A total of 6 fluorescent pseudomonads (VPJU, VPJL, VPAU1, VPAU2, VPAU3 and VPAU4) were found during the crop period. All the isolates produced IAA and ammonia with varying degrees of intensity. Three isolates (VPAU1, VPAU3 and VPAU4) produced HCN, and no microbial isolates produced siderophore. The effect of soil temperature, pH, EC and carbon dioxide evolution was correlated with the number of fluorescent pseudomonads in the soil. The bacteria were significantly afflicted by pH and EC, whereas soil temperature and CO<sub>2</sub> evolution did not show any effect on the number of fluorescent pseudomonads. There was no significant influence of soil temperature, pH, EC and carbon dioxide evolution on indole acetic acid production, ammonia, and HCN production. Inoculated <i>Pseudomonas fluorescence</i> did not survive in <i>Pokkali</i> rice fields. However, further studies are needed for at least three seasons in <i>Pokkali</i> soils to confirm the results of the present study.</p>

### Introduction

Upadhyay *et al.* (2011) reported that saline-tolerant PGPR can alleviate soil salinity stress during plant growth and exopolysaccharide (EPS) secreted by the bacteria. Different bacterial genera have been proposed to alleviate salt stress in plants, such as *Agrobacterium*, *Arthrobacter*, *Azotobacter*, *Azospirillum*, *Bacillus*, *Burkholderia*, *Klebsiella*, *Pseudomonas* (Costa-Gutierrez *et al.*, 2020; Lami, *et al.* 2020), *Rhizobium*, and *Serratia*. Earlier studies have reported that the potential PGPR with plant growth-promoting traits should be able to

efficiently colonize the rhizosphere and survive under field conditions for effective use as bioinoculants (Amaya-Gómez *et al.*, 2020). Fluorescent *Pseudomonads* are the most promising plant growth-promoting rhizobacteria involved in the biocontrol of plant diseases, produce phytohormones, hydrogen cyanide (HCN), siderophores, indole-3-acetic acid (IAA), and antibiotics. Matsuguchi and Sakai (1995) reported that the total bacteria and gram-negative bacterial population did not change significantly but total

fluorescent pseudomonads increased in saline soils. Few species of *Pseudomonas* can survive under saline condition and improves the plant growth in maize, cotton seedling, and *Cicer arietinum* L. (Mishra *et al.*, 2010). Thus, traits related to bacterial fitness in the environmental settings in which they will be applied must be considered an intrinsic part of the selection process for PGPBs.

*Pokkali* rice cultivation in Kerala state is considered a sustainable system which is one of the most eco-friendly farming practices in the world. The term '*Pokkali*' refers to a saline-resistant rice variety that is cultivated once a year. The *Pokkali* fields are in low-lying areas which are waterlogged with saline conditions due to the influence of tidal action. Normally, a single-season crop is cultivated during the low salinity phase and it is followed by prawn farming throughout the high saline conditions. Since *Pokkali* cultivation is organic, the use of agrochemicals for plant growth promotion and disease management is not desirable. One of the alternatives for plant protection is the use of Plant Growth Promoting Rhizobacteria (PGPR). However, the population and functional efficiency of the Plant Growth Promoting Rhizobacteria are influenced by both abiotic and biotic factors which affect the performance. Among the abiotic stress factors, climatic, microclimatic and soil parameters could adversely affect the performance of the PGPR. The purpose of this study was to see how soil temperature and soil factors affected the number and efficiency of fluorescent pseudomonads in the rhizosphere of *Pokkali* rice.

### Material and Methods

The present study was aimed at the evaluation of soil temperature, pH and carbon dioxide evolution on the number and efficiency of fluorescent pseudomonads around the root system of *Pokkali* rice at Vytilla in Ernakulam district of Kerala. One plot was maintained as a control (without *P. fluorescens*), while another was treated by the most widely used *P. fluorescens* as PGPR. *Pokkali* rice was cultivated during the virippu season, which spans from May to October (2017). A talcbased formulation of *Pseudomonas fluorescens* (KAU reference culture) was applied as a seed treatment (10g/kg) and also soil application (2.5 kg/ha). From June to October (2017), the rhizosphere soils of *Pokkali* rice were sampled at periodic

intervals. The fluorescent Pseudomonads were enumerated by Vidhyasekaran and Rabindran (1996) using King's B and King's A media and in vitro analysis for IAA, ammonia, HCN (Bakker and Schipper, 1987), and siderophore synthesis was done at periodic intervals. Soil temperature, pH, EC and CO<sub>2</sub> (Chonkar *et al.*, 2007) evolution were all recorded simultaneously. The number of bacterial isolates, microclimatic and soil parameters were recorded and CD values were calculated using the software OP STAT. The square root transformations of soil temperature were carried out using one-factor analysis through OP STAT. Correlation studies were conducted to study the effect of soil parameters on the population of fluorescent pseudomonads. To study the effects of soil temperature and soil parameters on the functional efficiency of fluorescent pseudomonads, the software WASP 2.0 was used to cross-aggregate the data through the significance of chi-square statistics.

### Results and Discussion

The number of fluorescent pseudomonads around the root systems of *Pokkali* rice before the start of the experiment was absent in both treated and control plots (Table 1). However, one isolate (VPJU) was obtained from an untreated plot in June 2017 (30 DAS). Fluorescent pseudomonads were absent in June 2017, September 2017 and October 2017 in the treated plot. The highest population ( $3 \times 10^3$  cfu g<sup>-1</sup>) was recorded in August 2017 (90 DAS) and was absent from July 2017 to October 2017 in the control plot. Characterization of bacterial isolates for identification was studied on Kings' B agar medium. Based on the morphological, cultural and biochemical characteristics, all the isolates were tentatively assigned to the fluorescent pseudomonads. The isolates of fluorescent pseudomonads were screened for their functional efficiency at a monthly interval (Table 2). Among the six isolates (*Pseudomonas* sp (VPJU), *P. aeruginosa* (VPJL), *Pseudomonas* sp (VPAU1), *Pseudomonas* sp (VPAU2), *P. aeruginosa* (VPAU3) and *P. aeruginosa* (VPAU4) of fluorescent pseudomonads, all the isolates produced Indole acetic acid (IAA) and ammonia with varied intensity. Three isolates (VPAU1, VPAU3 and VPAU4) produced hydrogen cyanide (HCN) and

**Table 1: Total population of fluorescent pseudomonads in the Pokkali soils**

Month	Fluorescent pseudomonads (cfu/g)	
	T <sub>1</sub> (Control)	T <sub>2</sub> (PF)
Initial population of <i>P. fluorescens</i> in main field (May,2017)	ND	ND
June, 2017(30 DAS)	3.3×10 <sup>2</sup> (2.5)	ND
July, 2017 (60 DAS) (Second inoculation of <i>P.fluorescens</i> soil application)	ND	3.3×10 <sup>2</sup> (2.5)
August, 2017 (90 DAS and 30 DASI of <i>P. fluorescens</i> )	ND	3×10 <sup>3</sup> (3.5)
September, 2017 (120 DAS and 60 DASI of <i>P.fluorescens</i> )	ND	ND
October 2017 (150 DAS and 90 DASI of <i>P. fluorescens</i> )	ND	ND

( ): Logarithmic transformed values are given in the parenthesis

ND: Not detected

DAS: Days After Sowing

DASI: Days After Second Inoculation

**Table 2: Growth promotion and antagonistic traits of fluorescent pseudomonads.**

Month	Isolate code	Indole Acetic Acid (IAA)		Ammonia		Hydrogen (HCN)		Siderophore	
		T <sub>1</sub> (Control)	T <sub>2</sub> (PF)	T <sub>1</sub> (Control)	T <sub>2</sub> (PF)	T <sub>1</sub> (Control)	T <sub>2</sub> (PF)	T <sub>1</sub> (Control)	T <sub>2</sub> (PF)
June,2017	<i>Pseudomonas</i> sp. (VPJU)	+	a	++	a	++	a	-	a
July,2017	<i>P. aeruginosa</i> (VPJL)	a	+++	a	+++	a	-	a	-
August, 2017	<i>Pseudomonas</i> sp. (VPAU1)	a	++	a	+++	a	+++	a	-
	<i>Pseudomonas</i> sp. (VPAU2)	a	++	a	++	a	-	a	-
	<i>P.aeruginosa</i> (VPAU3)	a	+++	a	+++	a	++	a	-
	<i>P.aeruginosa</i> (VPAU4)	a	++	a	++	a	++	a	-
September 2017	ND	a	a	a	a	a	a	a	a
October 2017	ND	a	a	a	a	a	a	a	a

ND: Not Detected,

PF: *P.fluorescens*

a: No isolate obtained, - Negative, + Low, ++ Medium, +++ High

no isolates exhibited siderophore production. Isolate VPAU3 in August 2017 (90 SAR) followed by isolate VPJL in July 2017 (60 SAR) was the largest producer of IAA. The highest ammonia production was in the case of VPAU1 and VPAU3 isolate in August 2017 (90 DAS-Days after sowing ) followed by VPJL isolate in July 2017 (60 DAS-Days after sowing ) whereas, VPAU1 isolate in August 2017 (90 DAS) was the highest producer of hydrogen cyanide.

The mean monthly soil temperature recorded at two depths (5 cm and 10 cm) ranged from 27.3 to 33.3°C (Table 3). In the case of the treated plot, at 5 cm, the highest soil temperature (32.8°C) was in October 2017 (150 DAS) and the lowest (27.9°C)

in September 2017(120 DAS). However, at 10 cm depth, the highest soil temperature (32.0°C) was in July 2017 (60 DAS) and the lowest (27.3°C) in October 2017 (150 DAS). In the case of the control plot at 5 cm, the highest soil temperature (33.3°C) in July 2017 (60 DAS) and the lowest (27.6°C) in June 2017 (30 DAS). Whereas in the case of 10 cm depth, the highest soil temperature (32.1°C) in July 2017 (60 DAS) and the lowest (27.9°C) in September 2017 (120 DAS). Soil temperature was found to be significantly different in different months. But, soil temperature did not show any significant differences between the treated and control plot. Soil pH and EC and soil respiration were recorded in the rhizosphere at a monthly

**Table 3: Mean monthly soil temperature in Pokkali rice field at monthly intervals**

Treatment	Depth (cm)	Mean soil temperature (°C)									
		June'17		July'17		Aug'17		Sep'17		Oct'17	
		8:30 AM	2:30 PM	8:30 AM	2:30 PM	8:30 AM	2:30 PM	8:30 AM	2:30 PM	8:30 AM	2:30 PM
T <sub>1</sub> (Control)	5	27.6	29	29.6	33.3	30.5	31.6	27.9	31.1	28	32.5
	10	30	28.2	29.8	32.1	30.9	31.3	28	29.7	27.9	30.4
T <sub>2</sub> ( <i>P. fluorescens</i> )	5	28	29.6	30.3	32.6	29.2	31.3	27.9	30.8	27.4	32.8
	10	28.6	28.7	30	32	29.6	30.8	28	29.2	27.3	29.3
CD value (Treatment effect)	0.098										
CD value (Effect of soil temperature)	0.139										
CD value (Interaction effect)	0.196										
T <sub>1</sub> × T <sub>2</sub>	Probability of t statistic										
5cm (AM)	0.678 <sup>NS</sup>										
5cm (PM)	0.749 <sup>NS</sup>										
10cm (AM)	0.130 <sup>NS</sup>										
10cm (PM)	0.267 <sup>NS</sup>										

NS: Non-Significant

interval (Table 4, 5). The highest soil pH (7.45) in the treated plot was during October 2017 (150 DAS) and the lowest (6.28) was during August 2017 (90 DAS). The highest soil pH (7.23) was during October 2017 (150 DAS) and the lowest (6.17) was in August 2017 (90 DAS) in the case of the control plot. The highest soil EC (3.43 dS/ m) in the treated plot was recorded in July 2017(60 DAS) and the lowest (1.37dS/ m) was in August 2017 (90 DAS) whereas, the highest soil EC (3.02dS/ m) during July 2017 (60 DAS) and lowest (1.17dS/m) was in August 2017 (90 DAS) in case of control plot. The soil pH and EC were significantly different in different months and also between the treated and control plot. The highest soil respiration (16.28 mg CO<sub>2</sub>/g) in the treated plot was recorded in August 2017 and the lowest (1.54 mg CO<sub>2</sub>/ g) was in July 2017 whereas, the highest soil respiration (15.31mg CO<sub>2</sub>/ g) during September 2017 (120 DAS) and lowest (2.42mg CO<sub>2</sub>/g) in July 2017 (60 DAS) in the case of control plot. Carbon dioxide evolution was significantly different in different months and did not show any significant differences between the treated and control plot. The optimum growth of microorganisms is enhanced by a set of optimum environmental conditions (Pettersson, 2004). Correlation studies of soil temperature, pH, EC and carbon dioxide production with the number of fluorescent pseudomonads revealed that the population was negatively correlated with EC and pH (Table 6). Other parameters did not show any

effect on the number of fluorescent *Pseudomonas* sp. In a similar study, Gamliel and Katan (1990) found an inverse relationship between soil pH and the population of fluorescent pseudomonads. The effect of soil temperature, soil pH, soil EC and carbon dioxide evolution was correlated with the functional efficiency of fluorescent pseudomonads. The effect of soil temperature on IAA, ammonia, and HCN production did not show any significant differences. Maximum growth promotion activities of fluorescent pseudomonads were observed in a soil temperature range of 29.2 to 32.6°C. These results conform to the findings reported by Koche (2012) and Sarwaret *al.* (1992). The effect of soil pH on IAA, ammonia and IAA production did not show any significant differences. The soil pH ranged from 6.72 to 7.45. Earlier studies reported that maximum proliferation of the *Pseudomonas fluorescens* occurred at 30°C and 7.5 pH, whereas the growth of bacteria at lower pH was slower than in soils with neutral pH (Ambardar and Sood, 2010; Baath, 1998) which is contradictory to the present studies, in which maximum growth and growth promotion activities of fluorescent pseudomonads were in a soil pH of 6.28 to 6.89. In a similar study, Baath and Arnebrant (1995) reported that when pH changes from pH 4 to 7, a five-fold increase in bacterial growth were observed. In the present study, soil pH was almost neutral ranging from 6.17 to 7.45, which is favorable for the growth of fluorescent pseudomonads. Pokkali soils are acidic saline and the salinity of the soil might be the

**Table 4: Soil pH and soil EC in the rhizosphere of Pokkalirice at monthly intervals**

Month	Soil pH		Soil EC (dS/ m)	
	T <sub>1</sub> (Control)	T <sub>2</sub> ( <i>P. fluorescens</i> )	T <sub>1</sub> (Control)	T <sub>2</sub> ( <i>P. fluorescens</i> )
June,2017	6.72	6.86	2.8	3.24
July,2017	6.75	6.89	3.02	3.43
August, 2017	6.17	6.28	1.17	1.37
September, 2017	7.22	7.28	1.51	1.67
October, 2017	7.23	7.45	1.62	1.72
Probability of t statistic	2.486*		2.489*	
CD value	0.096		0.204	

\*Significant at 5% level

**Table 5: Carbon dioxide evolution in the rhizosphere of Pokkali rice**

Month	Soil respiration (mg CO <sub>2</sub> /g)	
	T <sub>1</sub> (Control)	T <sub>2</sub> ( <i>P. fluorescens</i> )
June,2017	3.34	1.76
July,2017	2.42	1.54
August, 2017	12.70	16.28
September, 2017	15.31	15.79
October, 2017	12.72	10.53
Probability of t statistic	0.837 <sup>NS</sup>	
CD value	1.347	

**Table 6: Correlation of micro-climatic and soil parameters with the number of fluorescent pseudomonads**

SN	Parameter	Correlation coefficient
<b>1.</b>	<b>Microclimatic parameter</b>	
a.	Soil temperature at 5cm (FN)	0.229 <sup>NS</sup>
b.	Soil temperature at 5cm (AN)	0.028 <sup>NS</sup>
c.	Soil temperature at 10cm (FN)	0.358 <sup>NS</sup>
d.	Soil temperature at 10cm (AN)	0.147 <sup>NS</sup>
<b>2.</b>	<b>Soil parameters</b>	
a.	Soil pH	-0.486*
b.	Soil EC	-0.223 <sup>NS</sup>
<b>3.</b>	<b>Microbial activity</b>	
a.	Carbon dioxide evolution	0.294 <sup>NS</sup>

**Table 7: Effect of fluorescent pseudomonads on plant height (cm) at monthly intervals**

Treatment	Plant height (cm)				
	June, 2017	July, 2017	August, 2017	September, 2017	October, 2017
T <sub>1</sub> (Control)	29.73	53.3	92.5	126.2	127.0
T <sub>2</sub> ( <i>P. fluorescens</i> )	30.58	55.5	92.7	134.6	135.1
CD value (Treatment effect)	14.243				
CD value (Plant height effect)	14.243				
CD value (Interaction effect)	28.486				
Probability of t statistic	0.868 <sup>NS</sup>				

Mean of 10 replication

**Table 8: Effect of fluorescent pseudomonads on the number of tillers in Pokkali rice**

Treatment	Number of tillers				
	June, 2017	July, 2017	August, 2017	September, 2017	October, 2017
T <sub>1</sub> (Control)	3	23	47	37	31
T <sub>2</sub> ( <i>P. fluorescens</i> )	4	24	50	38	35
CD value (Treatment effect)	60.154				
CD value (Number of tillers)	85.071				
CD value (Interaction effect)	120.308				
Probability of t statistic	0.348 <sup>NS</sup>				

**Table 9: Effect of fluorescent pseudomonads on yield attributes of Pokkali rice at harvest**

Treatment	Number of panicles/ hill (mean of 10 replication)	Number of grains/panicle (mean of 10 replication)	Chaff (%)	Yield (kg/ha)	1000 grain weight (g)
T <sub>1</sub> (Control)	34	77	18.5	2560	30.4
T <sub>2</sub> ( <i>P. fluorescens</i> )	47	78	18.1	3230	34.2
Probability of t statistic	0.121 <sup>NS</sup>	0.952 <sup>NS</sup>	0.95 <sup>NS</sup>	-	-

reason for the absence of fluorescent pseudomonads under neutral pH. The soil EC ranged from 1.17 to 3.43 in the present studies which were in agreement with earlier studies of the low saline phase in the Pokkali field (Shylarajet *et al.*, 2013). The EC fluctuates in Pokkali soils depending upon tidal action and the dilution effects of rainfall. The impact of soil EC on IAA, ammonia, and HCN production did not show any significant differences. Rangarajan *et al.* (2001) reported that soil salinity affects the rhizosphere *pseudomonads* population which might be the reason for the non-survivability of *Pseudomonas aeruginosa*. The carbon dioxide production ranged from 1.54 mg /g/day to 16.28 mg g/ day<sup>-1</sup>. The effect of CO<sub>2</sub> evolution on IAA, ammonia, and HCN production did not show any significant differences in the present studies. The effect of fluorescent pseudomonads on the height of the plant and height of the plant and tillers were non-significant (Table 7, 8). However, the highest plant height (135.4) was noticed in October 2017 (150 DAS) in the treated plot. Whereas, the highest number of tillers (50) was in August 2017 (90 DAS) in the treated plot. In a similar study, Deshwal and Kumar (2013) found that all the *Pseudomonas* strains enhanced plant growth in rice plants. *P. fluorescens*(PW-5) produced maximum shoot, root and dry weight. The treatment of *Pseudomonas fluorescens* contributed to the growth of the plant in terms of height and number of tillers. Seenivasan, (2011) also described the positive influence of *Pseudomonas fluorescens* on growth parameters of rice such as plant height, base length, shoot weight, root weight and the number of tillers per hill. Similarly, Salamone *et al.* (2012) reported an increase in the number of tillers in rice plants treated with rhizobacteria that promote plant growth, which is in line with the present studies. Yield parameters such as panicles per hill, number of grain per unit of panicle, chaff percent, thousand-grain weight, and grain yield were

recorded at the time of harvest and grain yield were recorded at the time of harvest (Table 9). It was found that all these parameters were non-significant in both plots. However, panicles per hill, number of grains per panicle, thousand-grain weight and grain yield were higher in the treated plot. Elekhtyarthe (2015) reported that *Pseudomonas fluorescens* (PGPR *Pf*) enhanced the grain yield and its attributes such as number of panicles m<sup>-2</sup>, number of grains panicle, percentage of filled grains, 1000 grain weight, and grain yield. Using 16S rDNA sequencing, VPAJU, VPAU1, and VPAU2 isolates were identified as *Pseudomonas* sp. and VPAJL, VPAU3 and VPAU4 isolates were identified as *Pseudomonas aeruginosa*. The inoculated *Pseudomonas fluorescens* did not survive during the crop period.

### Conclusion

In the present studies, inoculated *Pseudomonas fluorescens* was not found during the entire period of rice growth in Pokkali rice fields. However, the population of other fluorescent pseudomonads was found instead of *Pseudomonas fluorescens* in the rhizosphere soil of Pokkali rice. The population of other fluorescent pseudomonads found in the Pokkali soil was negatively correlated with pH and EC where as, soil temperature and carbon dioxide did not affect the population of fluorescent pseudomonads. The efficiency of fluorescent pseudomonads was not affected by soil temperature, pH, EC and carbon dioxide evolution. Hence, the fluorescent pseudomonads could be a potential PGPR in the Pokkali rice. However, further studies are needed to confirm why *Pseudomonas fluorescens* did not survive in Pokkali soils.

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## Conflict of interest

The authors declare that they have no conflict of interest.

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