J. Mycopathol, Res, ***50(2)*** : *305-308 (2012)*

Effect of temperature , carbon and nitrogen sources on growth of *Pseudomonas fluorescens*

MINA D. KOCHE*, R. M. GADE AND U. R. SANGALE
Department of Plant Pathology, Dr. PDKV., Akola 444104, Maharasthra

Received : 15.11.2012 RMS Accepted : 24.07.2012 Published : 29.10.2012

Experiment was conducted *in vitro* to see the effect of temperature, carbon and nitrogen sources on growth of different isotates of *Pseudomonas fluorescens.* The isolates of *P. fluorescens* were inoculated in King's B broth and incubated at different temperature ranging from 4 to 45°C (i.e. 4°,10°,15°,20°,25°,30°,35°,40° and 45°) and growth was measured turbidometrically. The temperature, 30°C (1.770) was found suitable for the growth than other temperature tested after 48 hr of incubation. Mannitol, dextrose, starch and fructose were added individually in the medium to see carbon utilization by the isolates of *Pseudomonas.* It preferred carbon through mannitol (0.701) and fructose (0.670) and found to grow after 96 hr of incubation. Dextrose (0.416) as a carbon surce recorded less growth and it was not preferred by all isolates. Among the four nitrogen sources (*viz.*, ammonium nitrate, ammonium sulphate, ammonium chloride and urea), ammonium nitrate (0.930) supported maximum growth in all Pf isolates after 96 hr of incubation.

Key words : Temperature, carbon and nitrogen sources, *P. fluorescens*

INTRODUCTION

Pseudomonas fluorescens is a soil microorganism living in symbiosis with fungi and plant and is considered as a plant growth promoting rhizobacterium. It promotes the growth of the fungal hyphae, which provide plant roots with nitrogen and phosphate sources. For instance, it has been found that the total P content in plants inoculated with the arbuscular mycorrhical fungus *Glomus caledonicum* was higher in the presence than in the absence of *P. fluorescens.* Due to its high versatility with respect to carbon and nitrogen sources, *P. fluorescens* can be used for a broad range of applications. It can be used in a wide variety of organic compounds as carbon sources or as electron donors for energy generation and it has the ability to use nitrate or nitrite as terminal electron acceptor when oxygen is not available in its environment. Temperature influences the rate of growth, metabolism and morphological characters of the bacterium. *P. fluorescens* can thrive under wide range of temperature and there is minimum, optimum and maximum for each of the species and even for particular strain .In order to investigate the physiology of *P. flourescens,* aerobic cultivations have been performed on different temperature, nitrogen and carbon sources.

*Email : mdkoche@gmail.com

MATERIALS AND METHODS

Soil samples were collected from rhizosphere of citrus in Vidarbha region (Table 1). Thirty isolates were obtained through serial dilution using King's B medium. Colonies that showed fluorescens were selected and further purified. Out of thirty *Pseudomonas fluorescens* Pf only effective fourteen Pf isolates were selected for study.

Fourteen isolates of *Pseudomonas fluorescens* were inoculated in King's B broth and incubated different temperature ranging from 4° to 45°C (i,e 4°,10°,15°,20°,25°,30°,35°,40°, and 45°) to find out optimum temperature for the growth. The growth was recorded at the end of 48 hr incubation and optical density was measred with the help of spectrophotometer at 620 nm.

Carbon sources (*viz.*,mannitol, dextrose, starch and

fructose) and nitrogen sources (*viz.*, ammonium nitrate, ammonium sulphate, ammonium chloride and urea) were incorporated in basal media (Ashthana and Hawker's medium) on the basis of molecular weight and inoculated with fourteen isolates of *P. fluorescens*. Optical density was measured at the end of 24, 48 and 96 hr of incubation at 620 nm.

RESULTS AND DISCUSSION

An experiment was conducted to know the requirement of temperature for growth of different isolates and the data are presented in Table 2. Differences among the isolates were significant.4 temperature of 30°C was found to be favorable for maximum growth of Pf_1 (1.803), Pf_9 (1.803), Pf_{10} (1.723), Pf_{14}(1.966),Pf_{18} (1.899), Pf_{19} (1.915), Pf_{26} (2.004) and Pf_{30} (1.685), where as, 25°C supported maximum growth of Pf_5 (1.931), Pf_6 (1.985), Pf_{15}(1.482), Pf_{16}(1.755) and for Pf_{20} (1.793) and only one isolate P_4 (1.586) found to sustain in 35°C temperature for better growth. Less growth was recorded in case of all the isolates when incubated at 4°,10°,15°,20°,40°, and 45°C. Thus, 25°C and 30°C temperature supported excellent growth of all the isolates. All fourteen isolates differed significantly in their relative growth indicating that each isolate had an ability to grow at specific temperature. Anonymous (2007) stated that optimal temperature for growth of *Pseudomonas fluorescens* are 25-30°C.

Table 1 : *Pseudomonas fluorescens* isolates obtained from soil samples of Citrus orchards in Vidarbha region.

Location District	Soil samples collected	Isolates no
Akola	Akola	Pf_1
	Akola	Pf_4
	Akot	Pf_5
	Akot	Pf_6
	Akoli Jahagir	Pf_9
	Akoli Jahagir	Pf_{10}
Amravati	Warud	Pf_{14}
	Warud	Pf_{15}
	Warud	Pf_{16}
	Amravati	Pf_{18}
Nagpur	RFRS Katol	Pf_{19}
	RFRS Katol	Pf_{20}
	Savandri	Pf_{26}
	Nagpur	Pf_{30}

Present findings corroborate with these results. Similar observations also observed by Guillou and Guespin-Michel (1996).

The experiment was also conducted to study the ability of different isolates to utilize carbon as an essential element from different carbon sources and data are presented in Table 3. Mannitol was found to support the maximum growth in isolates Pf_9 (1.140) followed by Pf_{16}(0.958) and Pf_{18}(0.945). Least growth was observed in Pf_6 (0.250). Fructose was found to support the maximum growth in

Table 2. Growth of different isolates of *Pseudomonas fluorescens* using King's B broth at different temperatures at 48 hr

Isolates	Temperature Growth (OD at 620 nm)								
No.	4	10	15	20	25	30	35	40	45
Pf_1	0.515	0.674	0.773	1.339	1.735	1.803	1.347	1.054	0.850
Pf_4	0.254	0.324	0.54	1.021	1.400	1.576	1.586	1.304	0.855
Pf_5	0.262	0.319	0.645	1.383	1.931	1.915	1.671	1.371	0.956
Pf_6	0.136	0.188	0.264	1.305	1.985	1.855	1.715	1.234	0.642
Pf_9	0.452	0.573	0.869	0.895	1.408	1.830	1.376	1.055	0.812
Pf_{10}	0.547	0.585	0.796	1.264	1.492	1.723	1.611	1.134	0.821
Pf_{14}	0.785	0.804	1.064	1.565	1.417	1.966	1.808	1.256	0.931
Pf_{15}	0.498	0.569	0.570	0.987	1.482	1.201	1.055	0.839	0.525
Pf_{16}	0.298	0.392	0.566	1.378	1.755	1.656	1.380	1.056	0.843
Pf_{18}	0.465	0.515	0.586	1.003	1.793	1.899	1.422	1.020	0.953
Pf_{19}	0.256	0.333	0.430	1.296	1.840	1.915	1.565	1.042	0.863
Pf_{20}	0.356	0.462	0.758	1.578	1.793	1.756	1.758	1.137	0.803
Pf_{26}	0.750	0.921	1.099	1.366	1.731	2.004	1.920	1.504	1.063
Pf_{30}	0.195	0.234	0.496	1.187	1.639	1.685	1.455	0.832	0.825
Mean	0.412	0.492	0.662	1.255	1.672	1.770	1.548	1.131	0.839
S.E.(m)±	0.08	0.07	0.14	0.06	0.06	0.14	0.05	0.05	0.05
CD(P=0.01)	0.26	0.23	0.45	0.20	0.21	0.46	0.17	0.18	0.17

Table 3 : Effect of different carbon sources on growth of different isolates of *P. fluorescens,*

Isolates no	Growth (at 620 nm)											
	Fructose			Mannitol			Starch			Dextrose		
	24 hr	48 hr	96 hr	24 hr	48 hr	96 hr	24 hr	48 hr	96 hr	24 hr	48 hr	96 hr
Pf_1	0.392	0.552	0.730	0.265	0.356	0.417	0.410	0.448	0.926	0.83	0.464	0.496
Pf_4	0.204	0.269	0.362	0.183	0.352	0.664	0.099	0.163	0.168	0.125	0.161	0.233
Pf_5	0.166	0.316	0.436	0.242	0.304	0.543	0.231	0.272	0.401	0.340	0.436	0.478
Pf_6	0.156	0.286	0.376	0.099	0.191	0.250	0.077	0.105	0.185	0.111	0.146	0.206
Pf_9	0.709	0.837	0.981	0.730	0.861	1.140	0.326	0.418	0.72	0.306	0.346	0.368
Pf_{10}	0.554	0.788	0.986	0.334	0.521	0.805	0.391	0.454	0.505	0.398	0.511	0.528
Pf_{14}	0.234	0.438	0.581	0.395	0.463	0.628	0.263	0.283	0.288	0.064	0.146	0.151
Pf_{15}	0.258	0.271	0.485	0.139	0.194	0.675	0.085	0.110	0.134	0.069	0.109	0.159
Pf_{16}	0.549	0.734	0.947	0.446	0.523	0.958	0.548	0.651	0.803	0.461	0.564	0.603
Pf_{18}	0.333	0.631	0.854	0.264	0.399	0.945	0.273	0.419	0.597	0.371	0.223	0.488
Pf_{19}	0.335	0.467	0.559	0.389	0.456	0.829	0.324	0.403	0.409	0.301	0.434	0.499
Pf_{20}	0.347	0.520	0.616	0.364	0.428	0.601	0.411	0.519	0.701	0.376	0.381	0.446
Pf_{26}	0.403	0.457	0.643	0.310	0.397	0.522	0.511	0.534	0.631	0.498	0.519	0.596
Pf_{30}	0.256	0.513	0.817	0.337	0.465	0.836	0.563	0.594	0.698	0.274	0.472	0.573
Mean	0.350	0.506	0.670	0.321	0.422	0.701	0.322	0.384	0.494	0.291	0.351	0.416
S.E.(m)±	0.042	0.045	0.035	0.032	0.036	0.046	0.031	0.033	0.036	0.030	0.042	0.043
C D (P=0.01)	0.145	0.152	0.114	0.106	0.114	1.059	0.119	0.122	0.130	0.110	0.146	0.148

Table 4 : Utilization of different nitrogen sources by different isolatis of *P. fluorescence*

Isolates no	Nitrogen sources (Optical Density at 620nm)			
	Ammonium nitrate	Ammonium sulphate	Ammonium chloride	Urea
Pf_1	1.064	0.434	0.182	0.808
Pf_4	1.158	0.256	0.250	0.319
Pf_5	1.043	0.178	0.392	0.254
Pf_6	0.316	0.134	0.079	0.076
Pf_9	0.892	0.301	0.332	0.565
Pf_{10}	1.032	0.227	0.343	0.426
Pf_{14}	1.437	1.451	0.653	0.710
Pf_{15}	0.154	0.149	0.172	0.206
Pf_{16}	0.598	0.434	0.295	0.483
Pf_{18}	1.047	0.263	0.267	0.473
Pf_{19}	0.779	0.352	0.268	0.435
Pf_{20}	1.007	0.577	0.473	0.878
Pf_{26}	1.331	0.346	0.592	0.760
Pf_{30}	1.157	0.960	0.442	0.526
Mean	0.930	0.433	0.338	0.494
S.E (m)±	0.052	0.055	0.051	0.057
C D (P=0.01)	0.174	0.185	0.169	0.192

Pf_1, Pf_9, Pf_{10}, Pf_{16} and Pf_{18}. Isolate Pf_1 (0.926) showed the maximum growth in starch and Pf_{15} (0.134) showed the minimum growth. Dextrose was found to be less effective for supporting the growth and the optical density ranged between 0.151 to 0.603.

The isolates preferred carbon through mannitol and fructose . Dextrose as a carbon source recorded less growth and it was not preferred for growth of all the isolate. Maximum growth was observed in mannitol and fructose which might be due to the ability of the isolate to preferentially utilize carbon form these sources as compared to other sources. Jayaswal *et al.,* (1990) reported that the best source of carbon was mannitol for *Pseudomonas* strain RJ2. There findings are similar to the results of the present investigation.

Inorganic nitrogen also plays an important role in reproduction of bacteria. Data presented in Table 4 indicated that among different nitrogen sources ammonium nitrate supported maximum growth of all isolates after 96 hr of incubation. Isolates Pf_{14} exhibited (1.437) OD followed by Pf_{26} (1.331) and less optical density observed in Pf_{15} (0.154) in ammonium nitrate. Isolates Pf_1 (0.808) exhibited maxmum optical density in urea. In general minimum growth was obtained when nitrogen was provided through ammonium chloride i.e. between the range of 0.076 to 0.653.

Wide range of optical density among the isolate within the same source of nitrogen might have resulted in variability. *P. fluorescens* has different af-

finities towards the different nitrogen sources and it is known that ammonia is the preferred nitrogen source, inhibiting the utilization of other nitrogen sources (Betlach *et al.*,1981). Abouseoud *et al.* (2008) found that ammonium nitrate as a best nitrogen source for production of biosurfactant by *P. fluorescens*. Rosenthal (1974) observed ammonium ion as sole nitrogen source for multiplication of *Pseudomonas*. The present results also corroborate with previous finding.

REFERENCES

Abouseoud, A., Maachi, R. Amrane, A., Boudergua, S. and Nabi, A. 2008. Evaluation of different carbon and nitrogen sources in production of biosurfactant by *Pseudomonas fluorescens*. *Desalination* **223:** 143-151.

Anonymous, 2007. *Pseudomonas fluorescens*.Wikipedia.

Betlach, M. R., Tiedje, J. M. and Firestone, R.B. 1981. Assimilatory nitrate uptake in *Pseudomonas fluorescens* using N. *Arch. Microbiol.* **129:** 135-140.

Guillou, C. and Michel, J.F. Guespin. 1996. Evidence for two Domains of growth temperature for the Psychrotrophic bacterium *Pseudomonas fluorescens* MFO. *Appl and Environ Microbiology.* **62:** 3319-3324.

Jayaswal, K. Marcel, Radheshyam. Fernandez, A. and Schroeded, Raiph G. 1990. Isolation and Characterization of a *Pseudomonas* strain that restrict growth of various Phytopathogenic fungi. *Appl. and Environ. Microbiol.* **56:** 1053-1058.

Rosenthal, S.L. 1974. A simplified method for single carbon sources tests with *Psesudomonas* species. *J. Appl. Bact.* **37:** 437-441.