Available online at www.pelagiaresearchlibrary.com

Pelagia Research Library

Advances in Applied Science Research, 2013, 4(4):409-412

Pelagia Research Library

ISSN: 0976-8610
CODEN (USA): AASRFC

Isolation of phosphate solubilizing bacteria and total heterotrophic bacteria from river water and study of phosphatase activity of phosphate solubilizing bacteria

Dipak Paul and Sankar Narayan Sinha*

Environmental Microbiology Research Laboratory, Department of Botany, University of Kalyani, Kalyani, West Bengal, India

---

ABSTRACT

Water samples from the river Ganga at Tribeni, West Bengal, India were sampled monthly to enumerate total heterotrophic bacteria (THB) and phosphate solubilizing bacteria (PSB) between January 2011 and December 2011. THB was high in the month of March and PSB was high in the month of July. The population density of THB ranged from 4.84-5.84x10^4 cells/ml and that of PSB ranged from 2.14-2.72x10^2 cells/ml. A significant variation in the capacity to solubilize phosphorous by the isolates of PSB were noted. The phosphatse activity of the isolates exhibited that the strain RPSB6 (27.38 µmoles/g/h) had higher activity followed by the strain RPSB9 (20.92 µmoles/g/h).

Keywords: Phosphate solubilizing bacteria, Total heterotrophic bacteria, River Ganga, Phosphatase activity

---

INTRODUCTION

Phosphorus is regarded as one of the major nutrients needed for the living organisms involved in most of the physiological processes. It exists in nature in a variety of organic and inorganic forms, chiefly in either insoluble or in very poorly soluble forms. Phosphorus can also be considered a pollutant if the concentrations are high under specific environmental conditions. The addition of phosphorus as phosphate ion is one of the most serious environmental problems because of its contribution to the increased eutrophication process of natural water bodies. Phosphate solubilizing microbes solubilize insoluble phosphorus by producing various organic acids. However, phosphate solubilization ability of microbes in natural ecosystems may be different from that found under laboratory conditions [1]. Microorganisms distributed in the marine environments play an important role in the decomposition of organic matter and mineralization. Studies on the occurrence of phosphate solubilizing microbes in freshwater ecosystem especially in the riverine ecosystem of Indian subcontinent are lacking. The present study was undertaken to study in detail about the distribution and population density of total heterotrophic bacteria (THB) and the constituent phosphate solubilizing bacteria (PSB) from the Ganga river water at Tribeni, West Bengal, India were enumerated and PSB isolates were also screened for their activity under *in vitro* conditions.

MATERIALS AND METHODS

Sampling

Water samples were collected in each month from the river Ganga at Tribeni (23°00'56"N and 88°24'54"E), West Bengal, India for a period of one year (January-December, 2011). Water samples collected in sterilized McCartney bottles were transported to the laboratory in an icebox immediately after collection for further studies.

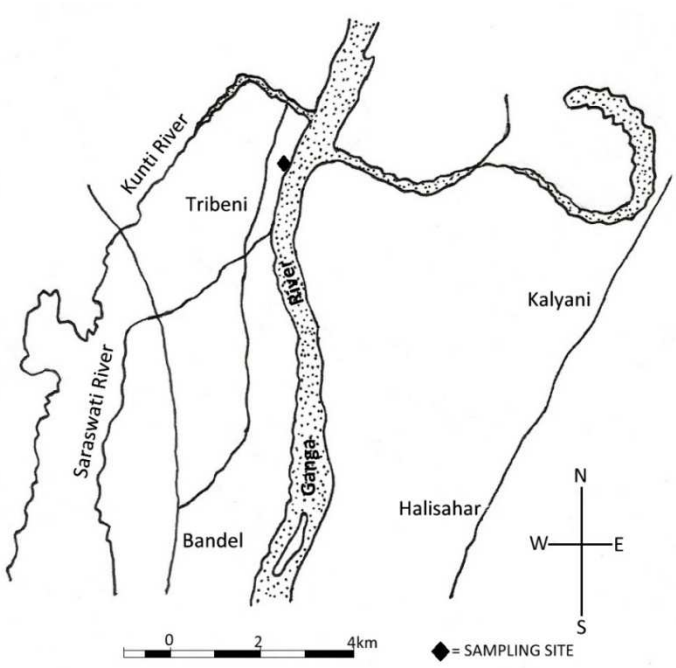

Fig 1. Map of the river Ganga showing sampling site

Determination of physicochemical parameters

Water samples were analyzed for the following physicochemical parameters; temperature, pH, redox potential (E_h), oxidation reduction index (rH_2), dissolved oxygen (DO) and phosphate. The physicochemical analysis of water samples were carried out in accordance with standard analytical methods [2].

Enumeration

Serial dilutions of the water sample were made and one ml of aliquots of 10^2-10^4 dilutions were transferred to Petri plates containing Nutrient Agar for enumerating THB and Pikovskaya's agar media for enumerating PSB. Plating was done in triplicate and incubated at room temperature ($28^0C \pm 2^0C$). After 48 h of incubation CFUs of THB were recorded and after 120 h of incubation CFUs of PSB were recorded.

Determination of phosphate solubilization

The phosphate solubilization potential of selected strains of PSB was estimated *in vitro* by determining available phosphorus in the Pikovskaya's medium amended or supplemented with tricalcium phosphate as substrate. The flasks were inoculated with bacterial culture broth (OD=2 at A_{600}). The flasks were incubated at 30^0C for 5 days and centrifuged at 15000 rpm. Phosphate was determined in supernatant as per the procedure outlined by Natarajan and Buvana [3]. Phosphate solubilization on solid medium was measured in terms of solubilization efficiency.

Percentage of solubilization efficiency= (Z-C)/C x 100

where Z= zone of solubilization, C= diameter of colony.

Determination of phosphatase activity

To study the phosphatase activity, experiments were set up using known bacterial broth cultures in flasks with and without adding phosphorous source (β-glycerophosphate used as a substrate). Culture filtrates were centrifuged and subjected to estimate phosphatase activity following the method of Tabatabai and Bremner [4].

RESULTS AND DISCUSSION

The physico-chemical parameters of the river water are presented in Table 1. The mean values of temperature fluctuated between 25^0C to 34^0C with the average value of 30.79^0C throughout the period of survey. Similarly pH of the river water oscillated between 3.50 and 4.14 with the mean value of 3.83. The redox potential (E_h) ranged between 0.738V and 0.807V with the mean value of 0.773V. The oxidation reduction index (rH_2) fluctuated between 33.74 and 34.84 with the mean value of 34.33. The dissolved oxygen (DO) value ranged between 0.90 mg/l and 1.80 mg/l with average value of 1.35 mg/l. Regarding phosphate concentration, it ranged from 0.976 mg/l to 0.723 mg/l with mean value of 0.853 mg/l.

Table 1: Physicochemical parameters of river water monitored during January-December, 2011

Month	Parameters					
	Temperature (^{0}C)	pH	E_h (V)	rH_2	D.O. (mg/l)	Phosphate (mg/l)
Jan	25.0	3.92	0.763	34.177	1.20	0.723
Feb	26.5	4.06	0.750	33.992	1.80	0.852
Mar	30.5	4.14	0.738	33.742	1.50	0.786
Apr	31.5	3.90	0.767	34.272	1.60	0.766
May	34.0	3.60	0.798	34.727	1.20	0.876
Jun	33.0	3.50	0.807	34.841	0.90	0.932
Jul	32.5	3.56	0.802	34.775	0.90	0.972
Aug	34.0	3.74	0.783	34.480	1.00	0.976
Sep	32.0	4.10	0.745	33.893	1.60	0.902
Oct	33.5	3.90	0.767	34.258	1.50	0.862
Nov	31.0	3.70	0.788	34.603	1..50	0.850
Dec	26.0	3.87	0.770	34.308	1.60	0.702

Population densities of THB and PSB in this site are presented in Table 2. It is generally observed that there was a significant difference of population density of THB and PSB. Throughout the year of study THB population remained almost between 4.84-5.84x10^4 cells ml^{-1} with the highest value during March and lowest during August. The mean population of THB was 5.27x10^4 cells ml^{-1}. The phosphate solubilizers recorded were less in number and are found to fluctuate between 2.14-2.72x10^2 cells ml^{-1}. This variation in the population of PSB might be attributed to many factors such as nutrients, pH, organic matter and some enzyme activities in water column.

Table 2: Total heterotrophic bacteria (THB) and phosphate solubilizing bacterial (PSB) count

Month	THB	PSB
Jan	5.48±0.03055	2.23±0.04583
Feb	5.54±0.01528	2.49±0.03786
Mar	5.84±0.02309	2.51±0.04583
Apr	5.32±0.02309	2.30±0.06429
May	4.96±0.02309	2.38±0.03055
Jun	4.60±0.04163	2.46±0.04000
Jul	4.68±0.04000	2.72±0.05292
Aug	4.84±0.03055	2.34±0.04163
Sep	5.26±0.02309	2.38±0.03055
Oct	5.72±0.04619	2.20±0.06000
Nov	5.68±0.05292	2.28±0.04163
Dec	5.34±0.04163	2.14±0.03055

THB= No. x 10^4 cells ml^{-1}; PSB= No. x 10^2 cells ml^{-1}

The phosphate solubilizing efficiency of isolated strains of PSB indicated that all the strains solubilized inorganic phosphate contents efficiently in the medium (Table 3). Among the 5 strains, RPSB6 (144.36 µg P ml^{-1}) was found as the best in solubilizing phosphates followed by RPSB9 and RPSB5. The phosphate solubilization efficiency in the solid media ranged between 50.00 and 83.33%. The results showed a wide range of variations in phosphate solubilization efficiency. Similar findings have been reported by many authors [5,6,7,8].

The phosphatase activity of the isolates showed that the strain RPSB6 had higher activity (27.38 µmoles/g/h) followed by RPSB9 (20.92 µmoles/g/h). The phosphatase activity was low in RPSB7, RPSB8 and RPSB5. However, there was a positive correlation (r=0.946) between phosphate solubilizing efficiency and phosphatase activity. This might be due to the availability of higher amount of phosphate in the medium and the ability of the strains to solubilize [8]. Temperature and pH caused a delay in the expression of phosphatase activity in all the isolates studied *in vitro* condition, although 32-37^{0}C was found to be much more congenial of all the isolates.

Table 3 Phosphorous solubilizing ability and phosphatase activity of PSB *in vitro* condition

Isolates	Available P (µg P ml^{-1})	Phosphatase activity (µmoles/g/h)	Phosphate solubilization efficiency (%)
RPSB5	123.18	18.85	62.50
RPSR6	144.36	27.38	83.33
RPSB7	113.72	15.84	50.00
RPSB8	121.76	17.65	60.00
RPSB9	129.92	20.92	75.00

Acknowledgements
The authors thankful to University of Kalyani for financial support to carry out the research.

REFERENCES

[1]P. Gyaneshwar, G.N. Kumar, L.J. Parekh, *World J. Microbiol. Biotechnol.*, **1998**, 14, 669-673.
[2] APHA, Standard Methods for the Examination of Water and Waste Water, 21th edition, American Public Health Association, Washington DC, **2005**.
[3]T. Natarajan, R. Bhvana, Practical manual-microbial interaction in soil. Tamil Nadu Agricultural University, Coimbatore, **2000**, 19-22.
[4]M.A. Tabatabai, J.M. Bremner, *Soil Biol. Biochem.*, **1969**, 1, 301-307.
[5]K.K. Kapoor, M.M. Mishra, K.Kukreja, *Indian J. Microbiol.*, **1989**, 29, 119-127.
[6]S. Singh, K.K. Kapoor, *Environ. Ecol.*, **1994**, 12, 51-55.
[7]P. Ponmurugan, C. Gopi, *African J. of Biotechnology*, **2006**, 5 (4), 348-350.
[8]M.K. Sahu, K. Sivakumar, T. Thangaradjou, L. Kannan, *J. Environ. Biol.*, **2007**, 28(4), 795-798.
[9]S.K. Barik, C.S. Purushothaman, Proceedings of National Symposium on Frontiers in Applied Environmental Microbiology, 11-13 Dec, Cochin, **1998**, 165-170.