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REGENERATION OF INORGANIC NUTRIENT IN SEDIMENT WATER INTERFACE BY *PSEUDOMONAS PERFECTOMARINA*

MD. ABDUL KARIM* AND KIMIO FUKAMI

Laboratory of Aquatic Environmental Science, Faculty of Agriculture, Kochi University, Otsu 200 Monobe, Nankoku, Kochi 783-8502, Japan

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Abstract

Pseudomonas perfectomarina was isolated from a eutrophic bottom environment and introduced to stimulate heterotrophic and decomposing activities. The bacterial cells re-suspension was added to the experimental system after absorbing with porous substrates. The strain enhanced net regeneration of dissolved inorganic nitrogen and inorganic phosphorus from the sediment by two to fivefolds at different temperatures. The bacteria *P. perfectomarina* enhance net regeneration of 83.20, 148.08, 127.58 and 193.31 µM dissolved inorganic nitrogen and 14.12, 13.24, 14.43 and 23.21 µM dissolved inorganic phosphorous from the sediment at 20, 23, 24 and 26°C, respectively. Results suggested that the application of *P. perfectomarina* with substrates would be promising for improvement of eutrophic sediment water interface during summer.

Introduction

The growth in aquaculture has led to an increase in the use of feeds applied to water for improving production. However, only a small portion of the total nutrient input is recovered as the harvest of cultured organism(1). Traditionally, the nutrient wastes, mainly in the form of ammonia, nitrite, nitrate, phosphorus and organic matter have been delivered into the surrounding environments without giving little thought to their holding capacity(2). The wastes lead the hypernutrification of the water column and sediment which in turn has been found to have serious ecological and economical impacts on the recipient ecosystems(3). This organic enrichment has also caused lowered productivity in farms and increased mortality of the cultivated fish(4).

Water-column nutrient recycling by bacteria and other heterotrophic organisms is a dominant process supplying nutrients to phytoplankton in photic zones of both marine and fresh water ecosystems. The contribution of bacteria in nutrient regeneration depends on the chemical composition and supply rates of available bacterial substrates, temperature, and food-web structure and dynamics(5). Dissolved organic nitrogen (DON)

*Author for correspondence: Department of Botany, University of Dhaka, Dhaka-1000, Bangladesh. <akarim@du.ac.bd>.

is actively released during photosynthesis and that low-molecular-weight compounds such as dissolved free amino acids are major substrates for bacteria[6]. Phosphorus is found in fish feeds and is broken down into a more useable form (phosphate) through decomposition. In most cases phosphorus and nitrogen contribute to eutrophication in a watershed by promoting growth of algae. Watershed resource manager focused on reducing the amounts of phosphorus and nitrogen in a watershed when attempting to improve water quality. The major problem is to accelerate this metabolism sufficiently to cope with the rate at which the contaminant is delivered to the environment.

The study site Uranouchi Inlet, Kochi prefecture, Japan is highly eutrophic due to unlimited practices of fish cages with high protein nutrient input. In summer season microorganisms are active at high temperature but due to low DO in the study site it could not show their activity properly. So, stimulate the heterotrophic activities during summer, using bacterial strain that's active at high temperature with low DO concentration may be a possible way. Considering that, we tried to isolate promising bacteria that can be active during summer at high temperature although the DO concentration is limited[7]. Thus the objectives of the present study were: (i) Isolation of indigenous bacterial strains possessing high metabolic activities and (ii) stimulation of inorganic nutrient regeneration during summer at high temperature with low DO concentration.

Materials and Methods

Sampling station is a eutrophic area practicing intensive fish farming in cages. The sampling point was in vicinity of one of the farms and the average depth near the sampling point ranged 16 - 17 m. Water samples were collected from bottom (16 m depth) environment with Niskin water sampler (5 l cap.), while sediment samples were collected by Ekman-Birge grab sediment sampler from surface sediment and kept into icebox. The study was carried out on May 10, June 21, July 5 and August 16, 2002.

For isolation of effective bacteria that might be active at bottom environment, authors collected bottom water and surface sediment sample from the study site. The fast growing bacterial strains isolated from the bottom water and sediment were 150. Considering temperature dependent growth pattern and metabolic activity of the strain finally *Pseudomonas perfectomarina* was selected to use for the improvement of the sediment-bottom water interface[8].

All experiments were conducted in one liter glass bottle, which was previously washed in 2N HCl for two days. Sediment-water system was prepared by adding 50 g (wet weight) of sediment into the bottle with one liter of sample water, where sample water was slowly added on to the sediment to avoid the profuse mixture and kept it for two hours to minimize the disturbance effects[9] and transparency of the overlying water of sediment-bottom water complex system could be assured by visual estimation[10].

Bacterial broth culture was centrifuged at 10000 rpm (Himac CR 21E, Hitachi) for 10 minutes at 10ºC. The supernatant was discarded and bacterial cells' plate was gently rinsed with filter-sterilized (0.22 µm) sample water at least five times in order to remove associated nutrients and re-suspended with the same filtered water by pipetting. Three ml of bacterial cell re-suspension was used as inocula and introduced into the treatment bottles absorbing with substrates. In the control bottles same amount of substrates were used. The glass bottles were incubated *in situ* in dark at temperatures 20, 23, 24 and 26°C during May, June, July and August, 2002, respectively for ten days. Dissolved oxygen concentration was monitored by YSI, Model No. 85/10 FT, at every sub-sampling occasion.

Bacterial activities were evaluated by the changes of inorganic nutrient regeneration within the sediment-water interface during incubation. The fluxes were calculated by considering the first slope of dissolved inorganic nutrients released during incubation.

Just after adding bacteria, 25 ml sub-samples were taken at the beginning of the incubation. At three days interval 25 ml sub-samples were pipetted aseptically from 2 cm above the sediment surface of each bottle without any disturbance of the system and preserved at –25°C temperature. Concentrations of dissolved inorganic nutrients in the sub-samples were measured by using automatic analyzer (Bran+Luebbe TRAACS 800).

Results and Discussion

For stimulation of the nutrient regeneration in sediment water interface, selected bacterial strain of *Pseudomonas perfectomarina* was added. During the incubation of sediment-water system collected in May and incubated at *in situ* at 20°C, concentration of DIN and DIP released into the overlying water increased after addition of bacteria more than without addition (Table 1). In the results, the net releases of DIN and DIP, within sediment-water interface in a control parts were 69.04 and 1.70 µM, respectively, while in the system of adding bacteria they were increased 152.24 and 15.82 µM, respectively (Table 1). Total amount of added bacteria was 28.39% of the natural bacterial abundance (Table 2). The DO concentration changed during incubation from 3.65 mg/l at the beginning to 2.64 mg/l at the end (Table 3). Result of the experiment with sample collected on June and incubated at *in situ* temperature 23°C has been presented in Table 1. The net releases of DIN and DIP supplemented with *P. perfectomarina* were 180.75 and 19.22 µM and those of control were 32.67 and 5.98 µM, respectively (Table 1).

Total amount of added bacteria was 27.75% of the natural bacterial population (Table 2). The DO concentration also changed from 1.83 - 0.38 mg/l (Table 3).

Table 1. Changes in concentration of inorganic nutrients by adding *P. perfectomarina* at different *in situ* temperature.

Months	Incubation temp. (°C)	Concentration of DIN (μm)											
		Without *P. perfectomarina*						With *P. perfectomarina*					
		0	2	4	6	8	10	0	2	4	6	8	10
May	20.0	43.03	51.58	73.53	86.72	97.49	112.07	43.01	98.92	143.53	164.60	181.02	195.25
June	23.0	69.65	79.33	83.18	87.27	99.42	102.32	80.4	140.28	171.93	223.22	247.51	261.15
July	24.0	55.25	67.09	69.71	78.65	84.85	89.61	63.24	123.68	156.35	184.29	213.59	225.19
Augst	26.0	66.56	82.78	95.74	106.62	122.1	127.53	69.81	176.55	249.97	277.03	303.81	324.1
		Concentration of DIP (μm)											
		Without *P. perfectomarina*						With *P. perfectomarina*					
		0	2	4	6	8	10	0	2	4	6	8	10
May	20.0	1.59	2.83	3.03	3.3	3.3	3.19	1.69	6.41	12.52	16.51	16.21	16.27
June	23.0	2.42	3.87	5.22	6.97	8.41	8.4	3.34	9.32	16.78	21.15	22.31	22.56
July	24.0	8.09	9.5	11.62	16.31	19.97	19.52	8.42	14.72	19.96	28.73	34.29	33.75
August	26.0	5.54	6.9	8.44	9.13	9.3	11.53	5.6	21.09	32.35	32.57	27.05	22.06

Simultaneously, experiments with samples of July and August with incubated *in situ* temperatures at 24 and 26°C, respectively have been conducted (Table 1). In the experiment of July, DIN concentration increased from 55.25 - 89.61 µM in control, while after addition of bacteria it changed from 63.24 - 225.19 µM (Table 1). Same trend also continued with the experiment of sample of August, changed from 66.56 - 127.53 µM in control, while after addition of bacteria it changed from 69.81 - 324.10 µM (Table 1). Accordingly, DIP concentration also increased from 8.09 - 19.52 µM without adding bacteria and after addition it changed from 8.42 - 34.29 µM in sample of July (Table 1). In the experiment of August, DIP concentration also increased from 5.54 - 9.30 µM without adding bacteria and after addition it changed from 5.60 - 32.57 µM (Table 1). Total added bacteria were 26.61 and 30.28% of the natural bacteria with sample of July and August, respectively (Table 2). The amount of DO also changed like before from 1.18 to 0.25 mg/l in sample of July and from 1.94 to 0.91 mg/l in sample of August (Table 3).

Table 2. Number of added bacterial cells and their ratio to natural bacterial abundances on different incubation occasions. Added bacterial stain was *P. perfectomarina.*

Months	Incubation temp. (°C)	Total bacterial abundance in sediment (×10^{10} cells)	Total added bacterial abundance (×10^{10} cells)	Added bacteria : Natural abundance (%)
May	20.0	46.5	13.2	28.39
June	23.0	47.2	13.1	27.75
July	24.0	48.1	12.8	26.61
August	26.0	49.2	14.9	30.28

Table 3. Changes in the concentration of dissolved oxygen (DO) at the initial and at the end of incubation on different incubation occasions. Used bacterial strain was *P. perfectomarina.*

Months	Incubation temperature (°C)	DO concentration (mg/l)			
		Without *P. perfectomarina*		With *P. perfectomarina*	
		Initial	End	Initial	End
May	20.0	3.90	3.14	3.65	2.64
June	23.0	1.73	1.43	1.83	0.38
July	24.0	1.16	0.99	1.18	0.25
August	26.0	1.84		1.94	0.91

The enhancement in the releasing rates of DIN and DIP after adding *P. perfectomarina* during experiment with samples collected in May, June, July and August are summarized in Table 4. During experiment in May, the releasing rates of DIN and DIP were 0.92 ± 0.03 and 0.13 ± 0.01 fM/d/cell, respectively while after adding bacteria it changed to 17.94 ± 2.23 fM/d/cell in DIN and 1.32 ± 0.02 fM/d/cell in DIP, respectively

(Table 4). In experiment with sample of June, releasing rates of DIN and DIP were 1.02 ± 0.01 and 0.15 ± 0.02 fM/d/cell, respectively while after adding bacteria it changed to 19.16 ± 2.11 and 1.73 ± 0.01 fM/d/cell, respectively (Table 4). Such enhancement were also noticed with samples of July, after adding bacteria DIN changes from 1.23 ± 0.05 to 18.98 ± 3.16 fM/d/cell and DIP changes from 0.15 ± 0.01 to 1.91 ± 0.01 fM/d/cell, respectively (Table 4). The same trend has also been observed in experiment with sample of August, releasing rates of DIN and DIP were 1.65 ± 0.04 and 0.17 ± 0.02 fM/d/cell, respectively while after adding bacteria it changed to 30.38 ± 2.17 and 4.64 ± 0.07 fM/d/cell, respectively (Table 4).

Table 4. Changes in releasing rate of DIN and DIP fluxes after addition of *P. perfectomarina* at different *in situ* temperature.

Months	Incubation temp. (°C)	Releasing rate of DIN (fM/d/cell)		Releasing rate of DIP (fM/d/cell)	
		Without *P. perfectomarina*	With *P. perfectomarina*	Without *P. perfectomarina*	With *P. perfectomarina*
May	20.0	0.92 ± 0.03	17.94 ± 2.23	0.13 ± 0.01	1.32 ± 0.02
June	23.0	1.02 ± 0.01	19.16 ± 2.11	0.15 ± 0.02	1.73 ± 0.01
July	24.0	1.23 ± 0.05	18.98 ± 3.16	0.15 ± 0.01	1.91 ± 0.01
August	26.0	1.65 ± 0.04	30.38 ± 2.17	0.17 ± 0.02	4.64 ± 0.07

Increased ammonium regeneration rates corresponding to increased numbers of bacteria were assumed to be caused directly by bacteria, whereas the absence of increased ammonium regeneration rates with increased particle density was assumed to indicate that most of the ammonium regeneration was done by organisms[11]. The increased rates of ammonium regeneration with increased added bacterial concentrations that we observed at several stations may indicate that bacteria themselves directly regenerate ammonium[12] or may reflect the increased food supply made available to the bacterial grazers that would in turn cause regeneration rates to increase.

Soluble organic compounds attached to the plant and sediment surfaces are mainly degraded by bacteria aerobically. The oxygen needed to support the aerobic process is supplied directly from the atmosphere via diffusion through the sediment or water-atmosphere interface and by oxygen leakage from macrophytic roots[13].

The strain *P. perfectomarina* enhanced net amounts and rates of inorganic nutrients (DIN and DIP) regeneration in overlying water column on the sample of May, when incubated *in situ* at 20°C (Tables 1 and 4). During this experiment, added bacterial densities were 28.39% of the natural bacterial abundance (Table 2). The results of the experiment conducted with samples collected in June incubated *in situ* at 23°C with added bacterial density 27.75% were also consistent with the experiment conducted in May (Tables 1, 2 and 4).

Two more experiments were conducted; one was done with the sample of July that was incubated *in situ* at 24°C (Table 4). In this case added bacterial abundance was 26.61% to natural abundance (Table 2). The next experiment was done with sample of August and incubated *in situ* at 26°C (Table 4) and during this experiment the added bacterial abundance was 30.28% (Table 2).

Results of the experiments conducted with strain *P. perfectomarina* and incubated at different *in situ* summer temperatures showed that this strain could be effective and promising for the stimulation of decomposing sediment organic matter at high temperature (20 - 26°C) with low DO concentration (1.18 - 0.25 mg/l).

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