Evaluation of Chitinolytic Bacteria from Two Aquatic Systems with Varied Trophic Structure

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Abstract: ***Chitin which is the second most abundant polymer in the earth after cellulose is the structural component besides being the outer skeleton in many organisms. Chitinases and N- acetyl glucosaminidases are the most common enzymes which help in degrading chitin. The present study was undertaken to evaluate the chitinolytic bacteria present in two different aquatic systems with different trophic status in Tiruchirappalli, Central Tamil Nadu. Results indicate that maximum number of chitinolytic bacteria was uniformly recorded in summer for both systems. Further, the percentage contribution of chitinolytic bacteria in terms of total heterotrophic bacteria was uniformly highest in presummer for both systems. While the less eutrophic system recorded 10 chitinolytic species, the eutrophic system recorded 13 species. There were similarities as well as uniqueness in the composition of chitinolytic species. Their presence in these systems reveals the significant role they play in degrading chitin in such water bodies.***

Keywords: Chitinolytic bacteria, Fresh water system, Trophic status, Season.

1. Introduction

Chitin is a homopolymer of β-1,4 linked N – acetylated glucosamine. It has a wide distribution and is the second most abundant polymer on earth after cellulose (Kirchner, 1995). It is the structural component of the cell walls of fungi and algae and the outer skeleton of insects and crustaceans being at the same time present in the exoskeleton of coelenterates, platyhelminthes, protozoans and molluscs (Mulisch, 1993; Herth, 1978; Knorr,1984; Gooday, 1990; Kapaun and Reisser,1995; Huang *et al.,* 1996; Schlegel, 1996; Suzuki *et al.*, 1998). Literature further reveals that the annual production and the steady state amount in the biosphere is of the order of 10^{12} to 10^{14} kg (Jeunioux and Voss – Foucart, 1991; Poulicek *et al*., 1991).

Chitinases and N- acetyl glucosaminidases are the basic enzymes that help in degrading insoluble chitin into its monomeric components. Reports suggest the presence of these enzymes in various organisms including bacteria, fungi, insects, plants and animals. Hence the main aim of the present study was to evaluate the number of chitinolytic bacteria from two different water bodies present in Tiruchirappalli, Central Tamil Nadu, India during different seasons of the year.

2. Material and Methods

The study was carried out in two different water bodies in Tiruchirappalli, India. One was Kulumani Pond located about 14 km away from Tiruchirappalli junction while the second was Vayalur Pond located about 10 km from Tiruchirappall junction.

2.1 Sampling

The surface water (10-20 cm of the surface) and water from over the sediments (10-20 cm of the sediment) were sampled at two stations. All samples were placed in an ice thermo insulated containers (the temperature inside was not higher than ±7^0C) and brought to the laboratory where they were immediately analysed. The material was sampled in winter / rainy season (September –December), presummer (January – April) and summer (May – July) during 2012 – 2013.

2.2 Heterotrophic Bacteria Number

The number of heterotrophic bacteria in the water and bottom sediments were determined by means of spread plate method, inoculating the material on iron – peptone agar medium according to Ferrer *et al.* (1963). The samples were diluted with sterile buffer water after Daubner (1976). After 6 days of incubation at 20^0C, the grown colonies were counted by converting the result into $1Cm^3$ of water or 1g fresh sediment matter.

2.3 Chitinolytic Bacteria Number

The number of chitinolytic bacteria in the examined samples were determined by means of spread plate, inoculating the material onto a medium containing the following components: peptone (peptobak) - 0.1g, iron sulphate - 0.1g, ammonia sulphate – 0.1g, iron gluconate – 0.1g, yeast extract – 0.1g, colloidal chitin – 7.0g of dry mass, agar, - 15.0g, tap water – $0.1dm^3$, pH 7.2 – 7.4. The colloidal chitin was prepared according to Lingappa and Lockwood (1962). After 14 days of incubation at 20^0C, the bright halo diameter around the colonies were measured to get information on the bacterial chitin decomposition ability. These strains were then isolated onto semi –liquid substrate containing colloidal chitin and kept in a fridge. They were inoculated onto a fresh semi – liquid medium every 2 months.

2.4 Identification of Chitinolytic Bacteria

The identification of the chitinolyic bacteria under study was done according to the pattern suggested by Shewan *et al.*(1960) and data published in papers by Hendrie (1968), Thornley (1968) and Holt *et al.* (1994).

3. Results and Discussion

The various morphometric and trophic characteristics of the two aquatic systems chosen for the present study are presented in Table 1. As evident from the table, the aquatic system (Vayalur pond) was pond to be highly eutrophic when compared to the other (Kulumani pond).

The bacterial counts of heterotrophic and chitinolytic bacteria recorded during the three different seasons of the year for both the water bodies are presented in Table 2.

As evident from the table, both the ponds recorded highest number of chitinolytic bacteria during the summer season (pond 1: 4.2 x10^{-3} ; pond 2:7.7 x 10^{-3}) and winter /rainy season (pond 1:2.4 x 10^{-3}; pond 2: 3.7 x 10^{-3}). A comparison between both the systems revealed that pond 2 which is more eutropic than the other recorded a higher chitinolytic bacterial count in all the three seasons.

A perusal of literature reveals that Brzezinska and Donderski (2006) while analyzing the chitnolytic bacteria in two lakes also recorded maximal chitinolytic bacteria to occur in summer. Earlier, Donderski and Brzezinska (2001) while analyzing the chitnolytic bacteria in the various lakes of Hawskie Lake District also reported the same phenomenon. Thus, the results obtained in the present study are in line with the observation made by others.

A comparison between the percentage contribution of chitnolytic bacteria and total heterotrophic bacteria (Table 2) also produces interesting observations. Eventhough the maximal chitinolytic bacteria were recorded uniformly in summer for both the aquatic systems, a percentage comparison in relation to total hetrotrophic bacteria reveals that chitinolytic bacteria recorded their highest percentage during the pre- summer season. In both the ponds in winter, the percentage composition of chitinolytic bacteria with regard to total heterotrophic bacteria was 37 (pond1) / 41(pond2) while in presummer, it increased to 46(pond1) / 49 (pond2) and in summer it declined to 41(pond 1) /46 (pond2). A persual of literature reveals that Donderski and Brzezniska (2001) and Brzezniska and Donderski (2006) while studying chitinolytic bacteria in various lakes also reported that even though maximal chitinolytic bacteria occurred during the summer season, chitnolytic bacteria in terms of percentage against chitnolytic bacteria recorded higher levels during the autumn season. As autumn season is absent in these parts of Tamil Nadu, this could probably be the reason why maximum chitinolytic bacteria in terms of percentage was recorded during the presummer season.

The increased amount of chitnolytic bacteria during this period can be attributed to increased useage of chitin as the source of carbon and nitrogen. Similar suggestions were also reported by Undryk (1991) and Brzezinska and Donderski (2006). The species composition of chitinolytic bacteria that occurred in both the ponds are presented in Tables 3 and 4. The less eutrophic pond (Kulumani pond recorded a total of 10 species (Table 3) while the eutrophic pond recorded a total of 13 species (Vayalur pond).

In the lesser eutrophic pond, while the presummer season recorded the presence of 9 species, the summer season recorded the presence of 8 species and the winter/ rainy season recorded all the 10 species. A closer perusal reveals that among the species present, *Pseudomonas aerugniosa* dominated in all the three seasons while the least dominant one was *Serratia* sp.

In the eutrophic pond, the presummer and summer season recorded the presence of 8 species while the rainy /winter season recorded the presence of 12 species. Here also, all the three seasons were dominated by *Aeromonas hydrophila* while the least dominant species was *Serratia*.
As to the presence of chitinolytic bacteria, literature reveals that the generic composition is similar to the composition of bacteria found in different environments. Thus, the presence of various chitinolytic bacteria recorded in the present study are in line with the observations made by other workers (Donderski and Brzezinska, 2001; Brzenzinska and Donderski, 2006; Schlegel, 2003; Clarke and Tracey, 1956; Huang *et al*., 1996).

A comparison between the two ponds reveals that there were certain similarities as well as uniqueness. While both the ponds showed the presence of chitnolytic bacteria, their composition was not exactly the same. While *Pseudomonas aeruginosa* dominated in one system the other system was dominated by *Aeromonas hydrophila;* nevertheless, the least document species in both the systems was *Serratia*. Which higher chitinolytic bacteria count and species diversity was recorded in the eutrophic pond, both the ponds recorded a higher percentage of chitinolytic bacteria in relation to heterotrophic bacteria uniformly in the presummer season. Nevertheless, the presence of chitinolytic bacteria in both the ponds reveals that they do play a significant role in the decomposition of chitin in such water bodies which are abundant in this part of the globe.

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Table 1: Shows the details of the freshwater ponds

S.No	*Details*	*Kulumani Pond*	*Vayalur Pond*
1	Area	0.32	0.44 ha
2	Maximum Depth (m)	4.0	5.0
3	PH	7.5	8.2
4	Total Phosporus (mg / dm^{-3})	0.73 – 1.3	1.25 – 1.4
5	Total Nitrogen (mg / dm^{-3})	1.7 – 2.1	1.5 – 2.5
6	Electrical Conductivity (ms / cm^{-1})	210 - 260	280 - 340

Table 2: Shows the details of the Bacterial load in the pond

	Kulumani Pond (Pond 1)			*Vayalur Pond (Pond 2)*		
Season	Heterotrophic x 10^5	Chitinolytic x 10^3	Percentage composition	Heterotrophic x 10^5	Chitinolytic x 10^3	Percentage composition
Winter	6.4	2.4	37	8.9	3.7	41
Pre Summer	7.8	3.6	46	11.4	5.6	49
Summer	10.2	4.2	41	16.7	7.7	46

Table 3: Percentage contribution of taxes on of Chitinolytic bacteria in Kulumani pond

S.No		*Winter/Rainy season*	*Pre Summer season*	*Post Summer season*
1	Aeromonas hydrophila	12	10	16
2	Bacillus firmus	5	9	0
3	Bacillus subtilis	8	7	13
4	Flavobacterium sp	12	8	7
5	Vibrio cholerae	4	0	0
6	Chromobacterium sp	9	6	10
7	Enterobacter aerogenes	12	9	14
8	Pseudomonas aeruginosa	29	35	24
9	Alcaligenes denitrificans	7	12	12
10	Serratia liquefaciens	2	4	4

Table 4: Percentage contribution of taxas on of Chitinolytic bacteria in Vayalur pond

S.No		*Winter/Rainy season*	*Pre Summer season*	*Post Summer season*
1	*Aeromonas hydrophila*	25	30	22
2	*Bacillus megaterium*	15	10	0
3	*Bacillus firmus*	10	0	0
4	*Bacillus cereus*	6	9	12
5	*Flavobacterium sp*	5	8	12
6	*Vibrio cholerae*	3	0	0
7	*Chromobacterium violaceum*	8	8	12
8	*Enterobacter aerogenes*	18	12	10
9	*Serratia marcescens*	2	4	2
10	*Arthrobacter simplex*	4	4	8
11	*Pseudomonas aeruginosa*	11	15	10
12	*Klebsiella aerogenes*	3	0	12
13	*Achromobacter sp*	0	0	4