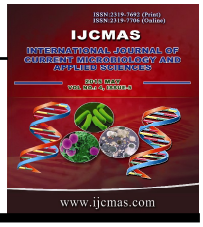
Int.J.Curr.Microbiol.App.Sci (2015) 4(5): 454-458

International Journal of Current Microbiology and Applied Sciences
ISSN: 2319-7706 **Volume 4 Number 5 (2015) pp. 454-458**
http://www.ijcmas.com



Original Research Article

A Comparison of Chitinolytic Bacteria and Their Enzymatic Activity in Two Freshwater Systems

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A B S T R A C T

Keywords

Chitinolytic, Bacteria, Cauvery River, Pond, Hydrolyse activity

Chitin is one of the main structural components of the outer skeleton of many organisms. Despite bacteria not containing chitin as a native component, numerous bacteria are able to hydrolyse chitin and use it as a carbon source. Hence the present study was attempted to identify chitinolytic bacteria of two aquatic systems. Results indicated the presence of 13 chitinolytic bacteria in the pond and 11 in the river system. Ten species were common to both the systems while two were unique to pond and one to the river system.

Introduction

Chitin is the second most common polysaccharide occurring in nature (Tsujibo *et al.*, 1998; Brurberg *et al.*, 2000) and is one of the main structural components of the outer skeleton of insects and crustaceans (Huang *et al.*, 1996; Schlegel, 1996) being at the same time the exoskeleton of coelenterates, platyhelminthes, protozoans, mollusks and in the cell wall of many fungi (Suzuki *et al.*, 1998). Chitinase is a hydrolase that degrades the chitin polymer, and like chitin, it is also present in a wide range of organisms (Someya *et al.*, 2011). In some cases, chitinases play a role in morphogenesis and/or autolysis in chitin – containing organisms (Brurberg *et al.*, 2000). Despite bacteria not containing chitin as a native component, numerous bacteria are able to hydrolyze chitin. Thus, it is conceivable that chitinolytic bacteria utilize chitin as a carbon source via the activity of chitinolytic enzymes.

Similar to other filamentous fungi, phytopathogenic fungi contain chitin as a main component of their cell walls. Therefore, chitinolytic bacteria show potential antagonistic activity against phytopathogenic fungi by degrading the cell walls of these fungi. In fact, it has been

reported that various chitinolytic bacteria such as those belonging to the genera *Aeromonas, Alcaligenes, Arthrobacter, Bacillus, Cytophaga, Lysobacter, Pantoea, Pseudomonas, Serratia* and *Stenotrophomonas* have the potential for the biocontrol of phytopathogenic fungi (Kobayashi *et al.,* 1995; Zhang *et al.,* 2001; Ramirez-Perez *et al.,* 2004; Someya *et al.,* 2004; Huang *et al.,* 2005; Kamil *et al.,* 2007; Park *et al.,* 2008). Thus the present study was aimed at identifying chitinolytic bacteria and their enzyme production from the aquatic systems.

Materials and Methods

The study was carried out in two aquatic systems - a fresh water pond (Naganathar pond) and a river (Cauvery) at Mukkambu - Upper Anicut area both at both at Tiruchirappalli, Tamil Nadu.

Sampling

The bottom water over the sediments was sampled in both the systems. All samples were placed in an ice thermoinsulated container (temperature inside was not higher than ± 7 °C), and brought to the laboratory where they were immediately analysed.

Chitinolytic bacterial analysis

The number of chitinolytic bacteria in the samples were determined by means of spread plates, inoculating the material onto medium containing the following components:– Peptone (Peptibak): 1.08 g; Iron sulphate: 0.1 g; ammonium sulphate: 0.18 g, Iron gluconate: 0.1 g; yeast extract: 0.18 g; Colloidal Chitin: 7.0 g of dry mass, agar: 15.0 g; tap water: 1.0 dm^3; pH: 7.2-7.4. The colloidal chitin was prepared according to Lingappa and Lockwood (1962). After 14 days of incubation at 20 °C, the bright halo diameter around the colonies was measured to get information in the bacterial chitin decomposition ability. These strains were then isolated into semi-liquid substrate containing colloidal chitin and kept in a fridge for later use.

Identification of chitinolytic bacteria

The identification of the chitinolytic bacteria was done according to the pattern suggested by Shewan *et al.* (1960), Hendrie (1968), Thornley (1968) and Holt *et al.* (1994).

Results and Discussion

The chitinolytic bacteria that were present in both the pond and river systems are presented in table 1. As evident from the table, a total of 14 species were recorded in both the systems. Among the two systems, Naganathar pond recorded 13 species while Cauvery River recorded 11 species. Further, 10 species were common to both systems while two were unique to Naganathar pond (*Alaligens denitrificans* and *Pseudomonas hydrophila*) and one to Cauvery River (*Bacillus firmus*).

The percentage composition between the two ponds reveals that in Naganathar pond, *Enterobacter* dominated the chytinolytic bacteria followed by *Aeromonas hydrophila* and *Bacillus subtilis* while in River Cauvery it was dominated by *Bacillus* followed by *Chromobacterium* and *Enterobacter aerogenes*.

Table 2 records the total chitinolytic bacteria present in pond and river water systems. A perusal of the table reveals that the pond ecosystem clearly recorded a higher number (2.2×10^4 cell cm^3) while the river system recorded lesser cells (1.4×10^4 cells cm^3).

Table.1 Percentage contribution of taxons of chitinolytic bacteria in two aquatic systems

S. No.	Bacteria	Pond System	River System
1.	*Aeromonas hydrophila*	14	11
2.	*Bacillus megaterium*	5	–
3.	*Bacillus firmus*	–	4
4.	*Bacillus cereus*	8	13
5.	*Bacillus subtilis*	11	2
6.	*Alcaligenes denitrificans*	5	–
7.	*Flavobacterium johnsoniae*	4	4
8.	*Chromobacterium* sp.	3	20
9.	*Vibrio* sp.	5	6
10.	*Enterobacter aerogenes*	25	12
11.	*Serratia marcescens*	7	15
12.	*Erwinia rhapontici*	4	6
13.	*Pseudmonas ichthyosis*	5	–
14.	*Pseudomonas aeruginosa*	4	7

Table.2 The numbers of chitinolytic bacteria (average) in two aquatic systems

	Pond System	River System
Surface water	1.2×10^4 cells $cm^3 \pm 0.5$	0.9×10^4 cells $cm^{-3} \pm 0.6$
Water	2.2×10^4 cells $cm^3 \pm 0.7$	1.4×10^4 cells $cm^{-3} \pm 0.5$

Table.3 Chitonolytic activity (mg–1 protein–1)

Days	River System	Pond System
1	0.8	1.4
2	3.8	4.6
4	5.8	7.0
6	7.4	8.2
8	6.2	7.8
10	5.8	6.5
12	4.4	5.6

Table 3 records the chitinolytic activity of the various bacteria in the two systems. As evident from the table, the chitinolytic activity was higher in the pond when compared to the river ecosystem. Nevertheless, both the systems recorded an

increase in chitinolytic activity with an increase in incubation time till the 6th day followed by a decline till the 12th day.

Thus there appears to be both similarities as well as uniqueness between the both the systems. Nevertheless, the pond aquatic system appears to show a higher chitinolytic bacterial diversity when compared to the River Cauvery. This might be attributed to not only the number of heterotrophic bacteria but also to the organic substances (protein, carbohydrate, fat, chitin, *etc.*) present in these systems. A comparison of the two systems reveals that the Naganathar pond is more eutrophic when compared to river ecosystem. This is undoubtedly related to the presence of organic substances which are more in the eutropic systems. Similar findings were also recorded by many authors (Donderski, 1984; Donderski and Brzezinska, 2001; Brzenzinska and Donderski, 2006) while working in various aquatic systems. The frequent occurrence of chitinolytic bacteria among planktonic bacteria may indicate the fact that the outer casing of many invertebrates containing chitin undergoes decomposition in lakes. The conditions are more favourable to ponds due to their occurrence, better oxidation and usually higher temperature (Donderski and Brzezinska, 2001).

The generic composition of chitinolytic bacteria is similar to the composition of bacterial community found in the different environments. There are many diverse taxons of bacteria which can decompose the chitin. The most commonly occurring chitinolytic bacterial species were *Aeromonas hydrophila, Bacillus cereus, Bacillus subtilis, Flavobacterium johnsoniae*, *Chromobacterium* sp., *Vibrio* sp., *Enterobacter aerogenes, Serratia marcescens, Erwinia rhapontici* and *Pseudomonas ichthyosis*. Literature reveals that Schlegel (2003) isolated a total of 50 bacterial taxons decomposing chitin from soil. In eutrophic lakes, Donderski and Brezezinska (2001) found *Achromobacter, Bacillus, Chromobacterium, Pseudomonas, Vibrio* and *Flavobacterium* to have chitinolytic activity; however, the most numerous genera were *Achromobacter* (49%), *Bacillus* (50%) and strains from the family of Enterobacteriaceae (48%) while Clarke and Tracey (1956) showed that numerous bacteria from the family of Enterobacteriaceae (*Klebsiella aerogenes, Erwinia* sp.) and bacteria from the genera *Vibrio* sp. and *Pseudomonas* sps. were capable of decomposing the chitin. Huang *et al.* (1996) also showed that bacteria from the genus *Aeromonas* were able to decompose the chitin. Thus, these observations are in line with the present study.

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