7.1 ENUMERATION OF ENTERIC BACTERIA IN MUGAIYUR LAKE OF CULTURED FISH

7.1.1 Introduction

India has been witnessing an overwhelming growth in the aquaculture sector for the past two decades and is presently ranked second in aquaculture production (Surendraraj et al., 2009). In freshwater culture, especially in India, carp culture plays an indispensable role contributing 93.6 per cent of the total freshwater production (FAO, 2005). While this growth has been much appreciated in terms of food security, today there is a growing awareness on the influence of bacterial composition of fish on the health and growth of the host as increased attention is been given to the possibility of cultured fish as vector of human pathogenic bacteria (Apun et al., 1999; Islam et al., 2000; Uddin and Al-Harbi, 2012). This is because, fish living in natural environments are been known to harbour a number of pathogenic organisms. It has also been observed that bacterial composition may change with age, individuals, nutritional status, environmental conditions and the complexity of fish digestive system (Cahill, 1990; Al-Harbi and Uddin, 2004; Ringo et al., 2006; Uddin and Al-Harbi, 2012).

The intestinal flora of organisms serves both digestive function and as a protection barrier against diseases (Sissons, 1989). The influence of gut flora on the host is clearly of great interest in aquaculture particularly where poor productivity and/or stock losses are widespread (Sharmila et al., 1996; Moriarty,
Seasonal Periodicity of Bacterial and Algal Diversity in Two Tropical Lakes

1997; Skjermo and Vadstém, 1999; Lavens and Sorgeloos, 2000). Further, the intestinal flora may be of significance in fish spoilage (Kaneko, 1971) and faecal contamination spread (Al-Harbi, 2003) and hence the present study.

7.1.2 Materials and Methods (Plate 9)

The lake from which fish were sampled was the Mugaiyur lake. Polyculture of carps was practiced only in the third year (2015) of study.

Water samples for microbiological analyses were collected, put aseptically into sterile 500 ml sampling bottles and examined within 1-2 hours of collection in the laboratory. All water samples were analysed for the presence of total and faecal coliform bacteria, faecal Streptococci and pathogenic *Salmonella* by the most probable number (MPN) method following the American Public Health Association (APHA 1980) procedures. The total viable count (TVC) of all heterotrophic bacteria was done on nutrient agar plates incubated at 28°C for 48 hours.

Ten specimens from each fish species were examined on the day of harvest. Swab samples of about 4-5 cm² fish skin area were collected and inoculated onto media as those used for the water samples to estimate the MPN values. Pieces of fish muscle, gills and digestive tracts were collected separately under aseptic conditions and put into sterile petridishes. Corresponding organs from the same fish species were pooled, weighed and homogenized with a sterile warring blender with 10 ml of 0.1% phosphate buffer saline of pH 7.5 per gram of fish tissue. A volume of 0.1 ml of the homogenate was plated subsequently onto nutrient agar
and Mac Conkey agar and incubated at 37 °C for 24-48 h. For qualitative identification of various bacteria from water and fish samples, fresh solid media of modified faecal coliform (M-FC) agar were inoculated in duplicate and incubated at 37 °C for 24 h. After distinct coloured colonies of various bacteria developed on the plates, further identification of the bacterial colonies were done according to Edwards and Ewing (1972), Cowan (1974), Martin and Washington (1980), Brenner (1984) and Cheesbrough (1989).

7.1.3 Results

Tables 29 and 30 present the size and weight ranges of the various carps used for the present study. All the fishes were almost of the same size and weight except for *Channa striatus* which was different in size and weight. Tables 31 and 32 record the bacterial counts in water and in various organs of the test fishes.

A comparison of the bacterial count in the skin among the four fishes reveals that *L. rohita* recorded the maximum count followed by *C. striatus*, *Cirrhinus mrigala* and *C. idella*. Amongst the gills, the maximum load was found in *C. striatus* followed by *C. mrigala*, *C. idella* and *L. rohita*. However, bacterial counts in intestine reveals that the maximum load was found in *C. idella* followed by *C. striatus*, *C. mrigala* and *L. rohita*.

Thus, in general, among the various organs analyzed, the maximum bacterial load was found in skin followed by gills and intestine for all the fishes.

The enteric bacteria isolated from the freshly caught fish were found to belong to 11 genera involving a total of 15 species. The bacterial species isolated
from water also belonged to the same 10 genera involving a total of 16 species. Thus, in general, the bacteria isolated from fish samples appeared to be very similar to those obtained from water. Among the various bacteria present in the fishes, *E. coli* and *P. aeruginosa* contributed the most (*L. rohita* - 34.34%; *C. striatus* - 18.2%; *C. mrigala* - 20.12% and *C. idella* - 18.3%). In the water also, the most dominant bacterial species was again *E. coli* (19.78%) and *P. aeruginosa* (15.44%). Thus, there appears to be a close correlation between the bacteria present in water and in organs of fishes with *E. coli* dominating followed by *P. aeruginosa* in all the four species of fish. A close perusal of the bacteria present in water and fish samples further reveals that all the bacterial species that were present in water was also found in all fishes even though the percentage was different. Thus among the various microbes *B. subtiles* was found in least counts in *C. idella* (0.14%) while it was *S. typhi* in *L. rohita* (0.20%). On the other hand, *Proteus mirabilis* was found in least number in *C. mrigala* (1.4%) and *P. vulgaris* in *C. striatus* (0.1%).

7.1.4 Discussion

Literature indicates that the intestinal bacterial flora from freshly caught fishes of other lakes also included *Enterobacter* sp, *Proteus* sp., *Salmonella* sp., *Pseudomonas* sp., *Klebsiella* sp., etc. (Souter *et al.*, 1976; Ogbondeminu, 1993; Sivakami *et al.*, 1996; 2011; Surendraraj *et al.*, 2009; Sankar Rao, 2013). These observations are in line with the present study.

Previous studies on the bacterial microflora of some fresh water fishes in tropical water (Fasanya *et al.*, 1988; Apun *et al.*, 1999; Islam *et al.*, 2000; Kumar
et al., 2001; Teophilo et al., 2002; Surendraraj et al., 2009; Sivakami et al., 2011; Sankar Rao, 2013) also showed that the most predominant organism isolated from the skin and gills of fishes belonged to the Enterobacteriaceae family as also reported in the present study. The bacterial composition in all the fish species appeared to be a reflection of the bacteria found in the lake water. Several authors (Geldreich and Clarke, 1966; Nieto et al., 1984; Buras et al., 1987; Ogbonde, 1993; Surendraraj et al., 2009; Sivakami et al., 1996, 2011; Sankar Rao, 2013) have also reported that the bacterial flora of fish is a reflection of their respective environments. Thus it is quite natural that *E. coli* and *P. aeruginosa* which are the dominant bacteria in water also dominated in all the fishes cultured. While the presence of *E. coli* can be attributed to the addition of animal manure, the entry of *P. aeruginosa* into the lake might be due to water containing these bacteria from the nearby areas entering into the system as surface runoff water. Thus, it appears that it is more likely to be a water related problem than a fish related one because the bacteria appears to enter the fish through the media. Nevertheless, the presence of a large number of enteric bacteria including pathogenic species in the system suggests the need for following strict hygienic methods during the process of handling and processing of fish in these systems. Besides, it also highlights the need for proper method before consumption of these fishes to prevent ourselves from getting diseases.

However, Al-Harbi and Uddin (2008, 2010) suggested that *A. hydrophila*, *S. putrefaciens*, *V. cholerae* and *Staphylococcus* sp. are the predominant isolates
while Choudhury et al. (1994) recorded *Micrococcus* and *Coryneforms* as the dominant bacteria in some fish systems.

In the present study, the bacterial composition differed in different fishes. According to Rheinhemer (1985), Al-Harbi and Uddin (2004) the composition and quantity of microorganisms vary depending on water temperature. In addition, fish habitat, age and conditions of the fish can also be some of the reasons for the differences in microbial composition in different fishes.

### 7.2 ANTIMICROBIAL STUDY USING SELECTED ALGAE

#### 7.2.1 Introduction

Biologically active compounds present in the plants have always been of great interest to scientists working in this field. In recent years this interest to evaluate plants possessing antibacterial activity for various diseases is growing (Krishnaraju et al., 2005; Raghavendra et al., 2006; Selvamaleeswaran et al., 2010; Haripriya et al., 2010).

Aquatic organisms are a rich source of structurally novel and biologically active metabolites (Ely, 2004). Secondary or primary metabolites produced by these organisms may be potential bioactive compounds of interest in the pharmaceutical industry.

Algae are a major source of food in the aquatic environment. It is commonly used as a food supplements to the humans and feed materials to the domestic animals. Further, it has been used as a source of biogas production and raw materials for the salad preparation (Sivakumar and Thanigaimalai, 2014).
Fresh water green algae are a group of fast growing autotrophic diversified organisms which transform radiant energy into chemical energy by capturing solar energy which maintain the homoeostasis of ecosystem and biomes. Besides having higher photosynthetic efficiency as compared to terrestrial plants, algae also have high growth rates and biomass production. Tropical conditions such as those in India provide favourable environment for the luxuriant growth of these organisms in the nature (Subbaramaiah, 1972; Srivastava and Odhwani, 1992; Thajuddin and Subramanian, 1992; Thajuddin et al. 2002; Rajakumar, 2004; Chellappa et al. 2004; Goyal S.K, 1962,1964; Bhatnagar and Bhatnagar 2005; and Bhatnagar et al. 2008; Makandar and Bhatnagar 2010).

Recent studies have shown that algae are rich sources of structurally novel and biologically active metabolites which are of interest in the pharmaceutical industry. The cell extracts and active constituents have been shown to have antibacterial activity \textit{in vitro} against Gram positive and Gram negative bacteria. In addition, a wide range of results of \textit{in vitro} antibacterial activities of extracts of fresh water as well as algae have been reported (Naik Ansari \textit{et al.}, 2012). Thus, plant based antimicrobials have enormous therapeutic potential as they can serve the purpose with lesser side effects that are often associated with synthetic antimicrobials. Hence, the present study was attempted in selected algae and cyanobacteria for antimicrobial activity.

**7.2.2 Materials and Methods**

**Sample Collection:** Water samples containing algae/cyanobacteria were collected from the Mugaiyur lake. Samples were isolated and identified by
standard microbiological methods (Desikachary, 1959; Rippka, 1988). To study the antimicrobial activity, algal and cyanobacterial species like *Anabaena aequalis*, *Lyngbya aestuarii*, *Oscillatoria angusta*, *Spirulina laxa* and *Synechocystis aquatilis* were selected.

Microorganisms like *Aeromonas hydrophila*, *Escherichia coli*, *Bacillus subtilis*, *Clostridium perfringens*, *Proteus vulgaris*, *Salmonella typhi*, *Streptococcus faecalis*, *Candida albicans* and *Aspergillus niger* were tested and obtained from Government Hospital, Tiruchirappalli. Bacterial strains were inoculated onto nutrient broth and incubated at 37°C for 24 hours. The fungal strains were also inoculated onto glucose peptone broth and incubated at 30°C for 5 days.

**Preparation of the algal extracts:** Ten day old algal and cyanobacterial cultures were collected, weighed and used for extraction of antimicrobial agents. 0.5 g of each of the five algal / cyanobacterial pellets were extracted in 10 ml of chloroform, diethyl ether, methanol and ethanol respectively. All extracts were preserved at 4°C (Gonzalez et al., 2001) for later use.

**Determination of the inhibition effect of the algal extracts:** Antimicrobial activities of cyanobacterial extracts were tested by agar well diffusion method. Nutrient agar plates were inoculated with 100 ml of a 24 hour broth culture of the test bacteria or 100 ml of a 5 day glucose peptone broth culture of the test fungi. Four wells (6 mm) were made and filled with 100 ml extract. The plates were incubated for 24 hours at 37°C for bacteria or incubated for 3 days at 30°C for fungi. The diameter of the inhibition zone was measured.
with calipers and the result recorded (Attaie et al., 1987). In addition, comparing the antimicrobial activity of cyanobacteria and algae with standard antibiotics (Amoxicillin and Polynoxylin) were also done.

7.2.3 Results (Plate 10)

The inhibition percentage of the antibacterial spectrum of methanol algal extracts are presented in Tables 33 and 34. As evident from the table, at a concentration of 0.5 mg/ml, the highest inhibition percentage against *E. coli* was shown by *L. aestuarii* extract (92.6%) followed by *A. aequalis* extract (75%) and the least by *S. trididemi* extract (0.4%). With regard to *S. typhii*, the maximum inhibition was shown by *O. angusta* extract (98.5%) followed by *S. laxa* extract (86.2%) and the least was by *L. aestuarii* extract (9.7%). In the case of *S. faecalis*, the maximum inhibition percentage was recorded by the extracts of *L. aestuarii* (94.7%) followed by *O. augusta* (93.3%) and the least by *S. laxa* (10.4%).

The percentage inhibition of water algal extracts are presented in Tables 33 and 34. As seen from the table, against *E. coli*, maximum inhibition was shown by *L. aestuarii* (74.4%) followed by *A. aequalis* (69.8%) and the least by *S. tridedemi* (0.1%) with regard to *S. typhi*, the maximum inhibition was recorded by *S. laxa* extract (94.2%) followed by *L. aestuarii* extract (90.1%) and the least by *O. augusta* extract (59.4%). In the case of *S. faecalis*, the maximum inhibition was recorded by the extract of *A. aequalis* followed by *S. laxa* (79.3%) while the least inhibitory rate was recorded by *L. aestuarii* (60.2%).

Thus, from the present study, it is clearly evident that all the algal extracts (both methanol and water) had inhibitory effect on the microbes even though the
percentage of inhibition varied. Among the methanol extracts, *L. aestuarii* extract showed maximum inhibitory effect against both *E. coli* and *S. faecalis* while it was *O. angusta* which recorded maximal inhibitory effect against *S. typhi*. However, among the water extracts, even though *L. aestuarii* again recorded maximal inhibitory effect against *E. coli*, it was *S. laxa* which recorded maximal inhibitory effect against *S. typhi* and *A. aequalis* extract against *S. faecalis*.

The results of the quercetin content is presented in Table-35. From the table it is clear that among all the algal species examined, the highest amount was found in *S. aquatilis* (42 mg/l) followed by *O. angusta* (32 mg/l) and the lowest in *A. aequalis* (4 mg/l).

Estimation of phycocyanin content also suggests that the maximum content was found in *S. aquatilis* (4.8 mg/l) and the lowest in *A. aequalis* (0.5 mg/l) (Table 35).

Determination of carotenoid content reveals that the maximum was also noticed in *S. aquatilis* ($3.3 \times 10^8$ mg/l) while the minimum in *O. angusta* ($7 \times 10^7$ mg/l) (Table-35). Details of chlorophyll-a content are provided in Table-35. As evident from the Table-35, *A. aequalis* and *O. angusta* continued to grow upto 16 days with maximum chlorophyll-a content reaching 7728.90 and 3991.1 $\mu$g/l followed by the stationary phase. With regard to *L. aestuarii* chlorophyll-a content at maximum growth phase amounted to 3172.30 (6th day) while the same for *S. taxa* was 2521.9 $\mu$g/l (10th day). Thus from the above data, it is clear that it can be concluded that the maximum biomass measured in terms of chlorophyll-a content as well as growth stages differed from each other.
The total carbohydrate and protein content present in each algal species are presented in Table-35. Among the five species, the maximum carbohydrate content was recorded by *S. aquatilis* (3.52 mg/l) followed by *A. aequalis* (2.12 mg/l) and the least by *S. laxa* (0.85 mg/l). On the other hand, the maximum protein content was recorded in *L. aestuarii* (0.66 mg/l) followed by *A. aequalis* (0.48 mg/l) while the lowest was in *S. laxa* (0.27 mg/l).

7.2.4 Discussion

Microalgae constitutes one of the commercially important living and renewable resources. They contain more than sixty trace elements including minerals, proteins, iodine, bromine and many bioactive substances (Asthana et al., 2009). To date, many chemically unique compounds of fresh water origin with various biological activities have been isolated (Choudhary et al., 2005; Parekh and Chanda, 2007; Abedin and Hala, 2008; Desbois et al., 2008; Kamble and Chavan, 2010; Elsie and Phanarajan, 2010) and some of them are under investigation while some are being used to develop new pharmaceuticals (Limafilho and Carvalho, 2002). It was also reported that the phenolic content are active as antibacterial against different types of microorganisms like *Salmonella typhi* (Ouattara et al., 2011) and the flavonoids are reported that they are active against several strains like *Streptococcus* (Shu et al., 2011), *E. coli* and *Staphylococcus aureus* (Gao and Zhang, 2010).

Sabarinathan and Ganesan (2008) evaluated the antibacterial effect of Phycocyanin pigment and proved its safety. Results of present study showed that
the Phycocyanin content of *Synechocystis aquatilis* was the highest (4.8 mg/ml) in comparison with other algal species.

It was also reported the *E. coli* and *Staphylococcus* are sensitive to polysaccharides (Li-Ya and Chang-Hong, 2010) and *S. aquatilis* showed the highest content of carbohydrates (3.52 mg/l).

The results showed that methanol extract of the selected algal species had inhibitory activities against gram-positive bacteria. Tuney *et al.* (2006) also showed that the methanol extract of *Gracilaria gracilis* exerted inhibitory effects against gram-positive bacteria *Streptococcus epidermidis* at a concentration of 25 μl. The antimicrobial activity of *Trichodesmium erythraeum*, a genus of filamentous cyanobacteria, showed an inhibitory effect against gram-positive bacteria *Enterococcus faecalis* and *Bacillus subtilis* at a concentration 0.315 μg/ml (Kasinathan *et al.*, 2009).

Umamaheshwari *et al.* (2009) found that methanol extract of *Halophila ovalis* exerted antibacterial effects against *Salmonella typhi* and *Salmonella paratyphi-B*. The results of Goud *et al.* (2007) showed that methanol extracts of several species of freshwater algae including *Nostoc* sp., *Lyngbya* sp., *Anabaena* sp. and *Mougeotia* sp. exerted antibacterial activity against Gram-negative bacteria *Salmonella typhimurium*. In contrast, methanol extract from other freshwater microalgal species such as *Pharmidiun* sp., *Cladophora* sp. and *Oscillatoria* sp. showed no inhibitory effects against *Salmonella typhimurium* at concentration 50 mg/ml (Abdo *et al.*, 2012).
The effect of water extracts of the selected algal species showed antibacterial activities against bacterial strains. It is clear that water extracts showed inhibitory effects lower than that of methanol. These results are in harmony with the finding of Goud et al. (2007), Sethubathi and Prabu (2010) and Abdo et al. (2012). From the present results it could be concluded that the antibacterial activity of the algae depends on the content of quercitin and phycocyanin pigments for the alcohol extracts and polysaccharide content for the water extracts of the species and the type of bacterial strains. From the present study, it is clear that *S. aquatilis* can be used for management of gram-positive and gram-negative infections.

### 7.3 IDENTIFICATION OF CHITINOLYTIC BACTERIAL LOAD IN TWO LAKE SYSTEMS

#### 7.3.1 Introduction

Chitinases class of enzyme which plays an important role for the degradation of chitin, the most abundant organic nitrogen-bearing compound in nature. Chitin, a linear 1, 4-B linked polymer of N-acetyl-B-D-glucosamine is the second most abundant polymer in nature after cellulose (Brurberg et al., 2000). Chitin is a major structural component of the exoskeleton of insects and crustaceans and it occurs in the cell walls of a variety of fungi. In accordance with the abundance of chitin, chitin-degrading enzymes are found in a variety of organisms including those non-chitin bearers such as bacteria, plant and vertebrates (Chih-Min Wen et al., 2002).
Many bacteria and fungi contain chitinolytic enzymes to convert chitin into compounds (Metcalfe et al., 2002) in the soil ecosystem and contribute to the recycling of vital carbon and nitrogen resources. Actinobacteria are thought to degrade and penetrate the chitinouslyphal walls of phytopathogenic fungi through the secretion of chitinase and other antifungal compounds. Addition of chitin to soil increases the counts of bacteria in particular, the streptomycetes and chitin amendment has been used as a biocontrol measure in soil (Kong et al., 2001; Krsek and Wellington, 2001).

Chitinases play an important role in normal life cycle function such as morphogenesis and cell division. Plants produce chitinases as part of their defence against fungal pathogens and regulators of growth process. In arthropods, chitinases are used as signals of symbiosis, for ecdysis, etc. Microorganisms can also use chitinases to decompose nutrients or reproduce cells.

Recently chitinases have been receiving more attention from biologists. A wide variety of medical application of chitin and chitin derivatives has been reported over the last three decades. N,N-Diacetylchitobiose has been widely used as starting material for synthesis of biological active compounds. Chitinases promise to be safer pesticides and microbial biocontrol agents. Chitinase activity in human serum has recently been detected. Chitinases and products of chitin degradation are used in industry of food, as biopesticide, as mosquito control agent, as anti-inflammatory and anti-tumour drug.

Chitinase enzymes are potential insecticides and insecticide adjuvants. They work by attacking the chitin that exist in the exoskeleton of insects. The ability of
chitinosan to bind metals, combined with its flocculating properties, provide applications in treating drinking water, pools and various waste streams. Hence the main aim of the present study was to evaluate the chitinolytic bacteria from two lake systems of Villupuram District, Tamil Nadu, India.

7.3.2 Material and Methods

The study was carried out in two different water bodies in Villupuram District, Tamil Nadu, India. One was Mugaiyur lake located about 21 km away from Villupuram junction while the second was Tirukoilur lake located about 37 km from Villupuram junction.

**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 °C) and brought to the laboratory where they were immediately analysed. The material was sampled in winter / rainy season (September to December), pre-summer (January to April) and summer (May to July) during 2013-2014.

**Heterotrophic Bacteria Number:** The number of heterotrophic bacteria in the water was 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 °C, the grown colonies were counted by converting the result into 1 cm³ of water or 1 g fresh sediment matter.
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) - 0.1 g, iron sulphate - 0.1 g, ammonia sulphate - 0.1 g, iron gluconate - 0.1 g, yeast extract - 0.1 g, colloidal chitin - 7.0 g of dry mass, agar - 15.0 g, tap water - 0.1 dm³, 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 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 two months.

Identification of Chitinolytic Bacteria: The identification of the chitinolytic 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).

7.3.3 Results and Discussion (Plate 11)

The morphometric and basic physio-chemical characteristics of the two systems chosen for the present study are presented in Tables 36-39. As evident from the table, Mugaiyur lake was found to be highly eutrophic when compared to Tirukoilur lake.

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 Tables 38 and 39.
As evident from the table, both the systems recorded highest number of chitinolytic bacteria during the summer season (Mugaiyur lake: $9.2 \times 10^5$; Tirukoilur Lake: $7.3 \times 10^4$) and pre-summer season (Mugaiyur lake: $4.1 \times 10^5$; Tirukoilur Lake: $4.4 \times 10^4$). A comparison between both the systems revealed that Mugaiyur lake 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. Sivakami et al. (2014) while evaluating the chitnolytic bacteria in two freshwater ponds also recorded maximum chitnolytic bacteria during the post-summer season. Thus, the results obtained in the present study are in line with the observation made by others.

A comparison between the percentage contribution of chitinolytic bacteria and total heterotrophic bacteria (Tables 36 and 37) reveals that chitinolytic bacteria recorded their highest percentage during the pre-summer season followed by summer and rainy season in both the systems. A perusal of literature reveals that Donderski and Brzezniska (2001), Brzezniska and Donderski (2006) and Sivakami et al. (2014) while studying chitinolytic bacteria in various systems also reported that even though maximal chitinolytic bacteria occurred during the summer season, chitnolytic bacteria in terms of percentage recorded higher levels during the pre-summer/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 chitinolytic bacteria during this period can be attributed to increased usage of chitin as the source of carbon and nitrogen. Similar suggestions were also reported by Brzezinska and Donderski (2006) and Sivakami et al. (2014).

The species composition of chitinolytic bacteria that occurred in both the systems are presented in Tables 38 and 39. The less eutrophic Tirukoilur lake recorded a total of 8 species (Table 39) while the eutrophic Mugaiyur lake recorded a total of 12 species. Further in Tirukoilur lake, the pre-summer season recorded the presence of 7 species, the summer and rainy seasons recorded all the 8 species. A closer perusal reveals that among the species present, *Pseudomonas aerugniosa* as also *A. hydrophila* dominated in all the three seasons. In Mugaiyur lake, the summer season recorded the presence of 11 species, pre-summer 10 species and while the rainy /winter season recorded the presence of all 12 species. Here also, all the three seasons were dominated by *Aeromonas hydrophila*. Sivakami et al. (2014) also reported the dominance of *P. aerugniosa* and *A. hydrophila* to dominate in various systems of Tamil Nadu.

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 (Clark and
Chapter VII

Experimental Studies

Seasonal Periodicity of Bacterial and Algal Diversity in Two Tropical Lakes

Tracey, 1956; Huang et al., 1996; Donderski and Brzezinska, 2001; Schlegel, 2003; Brzenzinska and Donderski, 2006; Sivakami et al., 2013).

A comparison between the two systems reveals that there were certain similarities as well as uniqueness. While both the lakes showed the presence of chitinolytic bacteria, their composition was not exactly the same. While *Pseudomonas aeruginosa* dominated in one system the other system was dominated by *Aeromonas hydrophila*. Even though higher chitinolytic bacteria count and species diversity was recorded in the eutrophic lake, both the systems 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 systems clearly suggests that they play a significant role in the decomposition of chitin in such water bodies which are abundant in this part of the globe.

SDS-PAGE profile of the partially purified chitinase enzyme produced by four dominant species (*Pseudomonas aeruginosa, Aeromonas hydrophila, Bacillus firmus* and *Serratia marcescens*) indicated that the molecular mass of the identified chitinolytic microbes ranged between 32 KDa and 48 KDa. For *Aeromonas* sp. it was 38 KDa, for *Serratia* sp. also the chitinase with 40 KDa was noticed, while for *Bacillus* it was 42 KDa and for *Pseudomonas aeruginosa* the molecular weight recorded was 32 KDa.

Screening the chitinase enzyme from *Bacillus* strain, Chin Chin Wen et al. (2002) reported the molecular mass of 36.5 KDa while Vetrivel et al. (2001) reported that a purified extracellular chitinase enzyme of Streptomyces peucetisu
had a molecular mass of 42 KDa on SDS-PAGE and Sheng et al. (2002) reported that an extracellular enzyme secreted by *Bacillus brevis* had the molecular mass of 85 KDa on SDS-PAGE electrophoresis analysis. The variation in molecular mass of chitinase synthesized by the *Bacillus* sp. in the present study may be attributed to the variation in species or the nature of enzyme purity, *i.e.*, the partial purified chitinase used in the present study. Ueda et al. (2003) reported a chitinase enzyme with molecular mass of 70.39 KDa for *Aeromonas* sp. while Park Jae Kweon et al. (2001) have also reported a chitinase with molecular mass of 60.0 KDa for *Enterobacter* sp. on SDS-PAGE analysis. Pleban et al. (1997) however, reported that the molecular mass of extracellular chitinase for *Bacillus cereus* was 36 KDa. Two chitinase A and B with the molecular mass of 43 KDa and 45 KDa were reported by Union Arab (2000) from the Streptomyces albovinoceus S22. For further understanding of the chitinase enzymes produced by the chitinolytic microbes, experiments with purified enzymes are of highly essential.