8.1 Introduction

The physiology of digestion and the presence as well as the activity of the digestive enzymes were investigated in order to gain an insight into the efficiency of the digestive system. There are several reports available on digestive enzymes of the different regions of the gut on gastropods and other molluscan forms (Ferreri and Ducato, 1959). Likewise information on the gut microflora of marine fishes (Sera and Ishidu, 1972), silver carp (Henbry et al., 1988) and some edible crabs (Venkataswaran, 1981) are available. Krishnamoorthy et al., (1965) studied the role of microbes in the nutrition of some estuarine and marine bivalves. Vanajakumar (1980), Sudha (1981), Palaniappan (1982) and Devendran et al. (1986) have also described the digestive process and the involvement of the gut microflora in digestion in the estuarine and marine organisms. There is also lack of information on the toxicity of sugar mill effluent on the enzymes of the digestive system and the gut microflora of different regions of the gut with reference to the freshwater fish and in particular C. striatus.
Bacteria are ubiquitous organisms and inhabit almost all the ecological niches. Aquatic systems support a great variety of bacterial species and other microorganisms; some of these are pathogens and most others are saprophytic and commensals. As there are myriads of higher aquatic organisms, the bacteria have different ecological relationship with these organisms starting from inquilinism to extreme endoparasitism. The toxic effects of the microbial pathogens will result in diseases whose severity will depend on the infecting microbe. Some of the pathogenic microbes may reach the internal organs through other orifices such as genital apertures and cause disease of the concerned system. The microbial communities of the digestive tract play a complementary role in digesting food stuffs which cannot be tackled by the native enzymes of the host organisms. Symbiotic microbes are mostly common in herbivorous forms as the type of food is a broad spectrum of vegetable matter. The carnivores on the other hand, do not face such a problem, since they take more or less uniform type of materials as food. It does not mean that the carnivores are completely devoid of gut microflora. They too have microbes, which are symbiotic and complement to the native enzymes (Muthu, 1989). All these associations are mutually beneficial and such relationship should have existed from early evolutionary times. The bacteria of the digestive tract may play beneficial role in the normal digestive activities of animal.

The present study has been carried out with the following objectives.

- To know the changes in essential digestive enzyme bound in different region of alimentary canal of *C. striatus* during the exposure period in sugar mill effluent.

- In order to have an insight into the distribution of heterotrophic bacterial population of control and effluent-treated *C. striatus*, the present study was undertaken. The organs chosen for microbiological studies are the stomach, mid gut and hind gut.
8. 2 Materials and methods

8. 2. 1 Digestive physiology

For the present study, the digestive system of *C. striatus* exposed to 30.5% and 61% (1/3 and 2/3 concentration of LC$_{50}$ 96 hrs value) concentrations of UASB treated sugar mill effluent for 60 days was divided into 1) stomach 2) mid gut 3) hind gut. These parts were dissected out from fish treated one to two days earlier with antibiotics to eliminate digestion due to microbes, and weighed individually and homogenised with measured quantity of distilled water to prepare 1% and 10% aqueous extracts which were centrifuged at 3000 rpm for 15 minutes. The supernatant of each sample was used as the enzyme source for further study. In order to prevent bacterial contamination a few drops of toluene were added to the supernatant. A control (fish reared in 100% water) was also maintained in which the enzyme extract was boiled and substituted for the active extract.

8. 2. 2 Qualitative tests of digestive enzymes

8. 2. 2. 1 Test for Carbohydrases

The presence of carbohydrases was tested by the method of Graham (1932). One percent aqueous solutions of sucrose, maltose, lactose, glycogen, starch, agar-agar and filter paper were used as the substrates to detect the presence of sucrase, maltase, glycogenase, amylase and cellulase; 5 ml of the 1% substrates such as sucrose, maltose, lactose and glycogen and 5 ml of the enzyme extract were incubated for 3 hours at 30°C ± 2°C. In order to detect the complex polysaccharases, 5 ml of 1% solution of the substrate (starch, agar-agar and filter paper) was incubated with 5 ml of the enzyme extract at 30°C ± 2°C for 24 hours. Wherever the experiments indicated low enzyme activity due to dilution, 10% extracts were used and the experiments were repeated. This procedure was followed for all the parts of the digestive system and the results were recorded. The end products of carbohydrate digestion in the different substrates were tested qualitatively with Benedict’s and Barfoed’s reagent. The appearance of discolouration from blue (amylase), appearance of brown (maltase) and red precipitate (glycogenase) indicated the presence of carbohydrases. Proper controls were run by incubating the same substrates, with heat inactivated enzyme extracts for the same lengths of time under the same temperature.
8.2.2.2 Tests for proteases

The presence of the proteolytic enzyme was qualitatively demonstrated by Harrow et al., (1960) using photographic film that contains a coating of gelatin. Proteolytic enzymes act on the gelatinous surface of the film and make it transparent by digesting the gelatin. Alternatively, equal quantities of 10% enzyme extract and 1% gelatin were incubated in test tubes for 12 hrs and the resulting products were examined. Liquification of gelatin indicated the positive results.

8.2.2.3 Test for lipases

Lipolytic enzymes were qualitatively demonstrated following the method given by Agarwal (1963) and Ward (1966). Fresh whole milk and boiled whole milk, diluted at 1:50, were used as substrates. One ml of the enzyme extract and one drop of 0.04% aqueous bromothymol blue were added to 1 ml of milk and a drop of 0.2N NaOH was also added to make the reaction mixture pale blue. The mixture was incubated for 12 hours at 30°C ± 2°C. The change in colour of the mixture from blue to yellow indicated the presence of lipases in the extract. Enzyme extracts for the different tests were prepared from the control and effluent treated fish.

8.2.3 Microbiology of the digestive systems

The control and effluent treated fish were collected from the respective culture tanks for enumeration of total viable heterotrophic bacterial population. The fish samples were brought to the microbiology laboratory in living condition immediately after the collection of the fish. Samples of stomach, mid gut and hind gut were aseptically dissected out for microbiological studies. Dissection instruments and containers were always sterilised before use. Care was taken to avoid contamination of adjacent tissues and for this the instruments were blame sterilised after removing each tissue. The aseptically excised organs were placed in separate sterilised petridishes. Since it was assumed that the different parts of C. striatus harboured specific microbial communities, the microbial analysis was performed individually for stomach, mid gut and hind gut samples.
8.2.3.1 Preparation of culture medium

Pour plate method was employed to enumerate the total heterotrophic bacterial population of digestive tract of *C. striatus*. Nutrient agar of the following composition was used to enumerate the digestive tract microflora.

- Peptone - 5.0g;
- beef extract - 3.0g;
- yeast extract - 2.0g;
- NaCl - 1.0g;
- agar - 15.0g;
- distilled water - 1000ml (pH - 7.2 ± 0.1)

The medium was autoclaved at 15 lbs / sq. inch for 15 minutes.

8.2.3.2 Preparation of Serial dilution

99 ml and 9 ml blanks of distilled water were autoclaved at 15 lbs / sq. inch pressure for 15 minutes. Known weights of stomach, mid gut and hind gut samples were homogenised individually using a small amount of sterilised distilled water from the 99 ml sterile blank and then transferred to the remaining distilled water blank. Each of the homogenised samples was thoroughly mixed using a rotary shaker for ten minutes for uniform dispersion of bacterial cells. The debris of tissues settled at the bottom of the container and bacteria lay distributed in the water. Serial dilutions of the bacterial samples were prepared as follows: 1 ml of the sample of the homogenate from 99 ml (10^2 dilution) the homogenate was added to the 9 ml sterile blank and thoroughly mixed in a vortex mixer. One ml of this diluted sample was again mixed with 9 ml of sterile blank. This process of dilution was repeated till the required dilution was obtained. Dilutions upto 10^6 were thus prepared and used for plating.

8.2.4 Plating procedure and enumeration of total heterotrophic bacterial population

One ml of aliquots of appropriate dilutions was pipetted out into sterile petridishes and 15 to 20 ml of sterile nutrient agar medium were poured. The medium and the inoculum were thoroughly mixed using turn table and the medium was allowed to solidify. Duplicate plates were also inoculated and all the plates were incubated at 37°C using bacteriological incubator. The duration between the collection of samples and the plating was minimised and it never exceeded two hours. The number of bacterial colonies were counted after 72 hours of incubation. Petridishes with 30 to 300 bacterial colonies were selected and the total viable bacterial counts were done using Quebec bacterial colony counter. The bacterial populations were expressed as number of colony forming units (CFU) per gram sample.
8.2.5 Isolation and identification of the bacterial strains upto generic level

Morphologically dissimilar well isolated bacterial colonies were selected at random from the nutrient agar plates of stomach, mid gut and hind gut samples. The selected colonies were examined for their morphology and pigmentation, if any, and the selected colonies were sub-cultured in nutrient agar plates to check the purity of the bacterial strains. The pure bacterial cultures were again sub-cultured in nutrient agar slants and then stored in a refrigerator at 4°C. These cultures were periodically sub-cultured to maintain the potency of the cultures. Altogether there were 83 isolates from the control, 66 isolates from the fish exposed to 30.5% effluent and 76 isolates from the fish exposed to 61% effluent were isolated and identified upto generic level. The Gram Positive bacterial strains were identified by employing the scheme developed by Simudu and Aiso (1962) and for the identification of Gram-negative bacterial strains the schemes of Shewan et al., (1960) and Gilmour et al., (1976) were followed. The bacterial Bergey’s Manual of Determinative Bacteriology (Williams and Wilkins, 1989) was also referred in the identification procedure.

8.2.6 Physiological grouping of bacterial isolates

The bacterial strains isolated from stomach, mid gut and hind gut of C. striatus, control and exposed, were used for this study. The bacterial strains were streaked on selective agar petriplates such as starch agar, gelatin agar and Tween 80 agar. The plates were incubated for 48 hours at 37°C in a bacteriological incubator. After the colonies attained good growth, the various bacterial strains were tested for amylolytic, gelatinolytic, caseinolytic and lipolytic properties. The starch agar, gelatin agar, casein agar and Tween 80 agar plates were prepared by using media of following composition.

**Composition of starch agar**

Peptone - 5g; Beef extract - 3g; Soluble starch - 2g; Agar -15g;
Distilled water-1000 ml (pH - 7.5 ± 0.1)

The plates containing bacterial growth obtained by streaking in the starch agar plates were tested for amylolytic activities using Gram’s iodine solution of the following composition.

Potassium iodide - 2g; Iodine crystals -1g; Distilled water - 100 ml
The petriplates were flooded with 10 ml of Gram’s iodine solution. The hydrolysis of starch by amylolytic bacteria resulted in the formation of clear zones around the bacterial growth. These bacterial strains were identified as amylolytic forms. The unhydrolysed starch produced a blue colour with iodine solution. The number of bacterial strains positive to tests for amylolytic activity were noted.

**Composition of gelatin agar**

Peptone - 10g; Meat extract -10g; Gelatin - 4g; Agar - 15g

Distilled water-1000 ml (pH - 7.2)

The bacterial strains isolated from stomach, mid gut and hind gut of C. striatus were streaked on the gelatin agar plates. After attaining good growth, the bacterial strains were tested for gelatinolytic activity using mercuric chloride solution of the following composition.

Mercuric chloride - 15g; Conc. HCl - 2 ml; Distilled water - 100 ml

The petriplates were flooded with mercuric chloride solution. Gelatinolytic bacteria hydrolyse gelatin; the testing with mercuric chloride showed the formation of clear zones around each of the gelatinolytic bacteria. The unutilized gelatin formed a white precipitate with mercuric chloride solution. The total number of gelatinolytic bacterial isolates were noted.

**Composition of Casein agar**

Peptone - 10g; Beef extract - 10g; Casein or Skimmed milk - 30g;

Agar - 15g; Distilled water - 1000 ml (pH - 7.2 ± 0.1)

Hydrolysis of casein by caseinolytic bacteria was shown by the appearance of a clear zone around each of the caseinolytic bacterial strain. The total number of positive Caseinolytic bacterial strains was noted.

**Composition of Tween 80 agar**

Peptone - 10g; Calcium Chloride - 0.1g; Tween 80 - 10 ml; Agar - 15.20g;

Distilled water - 1000 ml (pH - 7.2 to 7.4)

Hydrolysis of lipid by lipolytic bacteria resulted in the appearance of opaque zone and production of Oleic acid around the edge of such bacterial growth. The number of lipolytic bacterial isolates was counted and noted.
8. 3 Results

8. 3. 1 Qualitative tests of digestive enzymes

The presence of the native digestive enzymes of *C. striatus* is shown in Table 8.1.

8. 3. 1. 1 Carbohydrases

The stomach, mid gut and hind gut of control *C. striatus* were found to be rich in digestive enzymes as there was high reaction of amylase. In the stomach sucrase and lactase were present moderately. The mid gut of control samples contained low and moderate activity of lactase and glycogenase. Maltase and cellulase were completely absent in all the three parts of the digestive parts in control *C. striatus*. In addition glycogenase was absent in stomach; lactase and glycogenase were absent in the extract of hind gut. Low activity of amylase, sucrase, lactase and glycogenase were found to be present in the stomach of the fish exposed at 30.5% effluent. Whereas maltase and cellulase activities were completely absent in the stomach of fish exposed to 30.5% effluent. The high activity of sucrase was recorded in the mid gut of the fish exposed to 30.5% effluent. Amylase and glycogenase activity were low and maltase, lactase and cellulase were absent in the mid gut of fish exposed to 30.5% effluent. The hind gut contained high activity of amylase and low activity of sucrase and glycogenase; but maltase, lactase and cellulase activities were absent in the fish exposed to 30.5% effluent concentrations.

Extract from stomach of *C. striatus*, exposed to 61% effluent showed poor digestive enzyme activity as there was feeble reaction to sucrase and lactase. In mid gut, except glycogenase (high) all other enzyme activities were found to be poor. The hind gut was also found to be poor in digestion and feeble reaction to amylase, glycogenase was noticed. Cellulase activities were absent in stomach; no maltase, lactase and cellulase activities were observed in mid gut; the absence of lactase and cellulase enzymes in the hind gut of fish exposed to 61% concentration effluent (Table 8.1) was noticed.

8. 3. 1. 2 Protease

In control fish all the three regions of digestive tract showed the presence of protease. The hind gut exhibited high protease activity. On the other hand low protease activity was detected in the stomach of the fish exposed to 61% concentration.
Fig. 8.1  Total viable counts of heterotrophic bacterial population in stomach (ST), mid gut (MG) and hind gut (HG) of *C. striatus*; control fish after 60 days.
Fig. 8.2  Total viable counts of heterotrophic bacterial population in stomach (ST), mid gut (MG) and hind gut (HG) of *C. striatus* exposed to 30.5% effluent concentration after 60 days.
Fig. 8.3  Total viable counts of heterotrophic bacterial population in stomach (ST), mid gut (MG) and hind gut (HG) of *C. striatus* exposed to 61% effluent concentration after 60 days.
Table 8.1 Qualitative assessment of native enzymes in three regions of control and treated *C. striatus* gut.

<table>
<thead>
<tr>
<th>ENZYME</th>
<th>STOMACH</th>
<th></th>
<th>MID GUT</th>
<th></th>
<th>HIND GUT</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0% (C)</td>
<td>30.5% (1/3)</td>
<td>61% (2/3)</td>
<td>0% (C)</td>
<td>30.5% (1/3)</td>
<td>61% (2/3)</td>
</tr>
<tr>
<td>CARBOHYDRASES</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Amylase</td>
<td>+++</td>
<td>+</td>
<td>-</td>
<td>+++</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Sucrase</td>
<td>++</td>
<td>++</td>
<td>++</td>
<td>+</td>
<td>+++</td>
<td>+</td>
</tr>
<tr>
<td>Maltase</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Lactase</td>
<td>++</td>
<td>+</td>
<td>+</td>
<td>++</td>
<td>+</td>
<td>++</td>
</tr>
<tr>
<td>Glycogenase</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>++</td>
<td>+</td>
<td>+++</td>
</tr>
<tr>
<td>Cellulase</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>PROTEASE</td>
<td>+++</td>
<td>++</td>
<td>+</td>
<td>++</td>
<td>++</td>
<td>++</td>
</tr>
<tr>
<td>LIPASE</td>
<td>++</td>
<td>+</td>
<td>+</td>
<td>+++</td>
<td>++</td>
<td>+++</td>
</tr>
</tbody>
</table>

+++ = very high, ++ = high, + = moderate, ± = very low, - = absent, C = control

Table 8.2 Distribution of Total Viable Heterotrophic bacterial Population in the stomach, mid gut and hind gut of control and exposed *C. striatus* (cfu/g).

<table>
<thead>
<tr>
<th>Source</th>
<th>Control</th>
<th>30.5%</th>
<th>61%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stomach</td>
<td>$86.43 \times 10^3$</td>
<td>$11.63 \times 10^3$ (13)</td>
<td>$9.85 \times 10^3$ (11)</td>
</tr>
<tr>
<td>Midgut</td>
<td>$26.00 \times 10^5$</td>
<td>$85.12 \times 10^5$ (32)</td>
<td>$76.34 \times 10^4$ (29)</td>
</tr>
<tr>
<td>Hindgut</td>
<td>$32.25 \times 10^6$</td>
<td>$13.64 \times 10^5$ (4)</td>
<td>$10.45 \times 10^5$ (3)</td>
</tr>
</tbody>
</table>

Values in parenthesis indicate percentage against control.
8.3.1.3 Lipases

High lipase activity was observed in mid gut and hind gut and it was moderate in stomach. In 30.5% effluent exposed fish, hind gut showed high lipase activity followed by mid gut and stomach. Lipase level was high in the mid gut of fish exposed to 61% effluent and the same was low / very low level in stomach and hind gut of the fish exposed to 61% of effluent.

8.3.2 Total viable count of bacteria

The total heterotrophic bacterial population of the stomach, mid gut and hind gut of control and exposed *C. striatus* is shown in Table 8.2 and Fig. 8.1, 8.2 and 8.3. The heterotrophic bacterial density in the digestive tract (stomach, mid gut and hind gut) of the control fish *C. striatus* varied between $86.43 \times 10^3$ cfu/g and $32.25 \times 10^6$ cfu/g gut contents. Among the various digestive parts investigated, the hind gut sample registered higher bacterial density and the lowest counts were observed in the stomach region. The bacterial population of the digestive parts of the fish exposed to 30.5% effluent followed the same pattern as observed in control fish (stomach $11.62 \times 10^3$ cfu/g; mid gut $85.12 \times 10^4$ cfu/g; hind gut $13.64$ cfu/g). Considerable reduction was observed in the bacterial density in the stomach, mid gut and hind gut samples of *C. striatus* exposed to 30.5% effluent. The fish exposed to 61% effluent showed further decrease in number of bacteria (Stomach $9.85 \times 10^3$ cfu/g; mid gut $76.34 \times 10^4$ cfu/g; hind gut $10.45 \times 10^1$ cfu/g). This tremendous decline in number is remarkable with regard to gut microflora. For instance the stomach of the fish exposed to 30.5% and 61% contained only 13% and 11%; mid gut contained only 32% and 29%; hind gut contained only 4% and 3% of the original population (Table 8.2 and Fig. 8.1, 8.2, and 8.3).

8.3.3 Generic composition of microflora

The generic composition of digestive tract microflora of *C. striatus* is shown in Table 8.3. The various bacterial genera of digestive tract microflora was primarily comprised of *Bacillus, Micrococcus, Vibrio, Pseudomonas, Achromobacter* and *Flavobacterium*. *Micrococcus* and *Flavobacterium* were present only in the stomach of control fish. Analysis of the bacterial flora in the fish exposed at 30.5% showed the presence of above said five genera as observed in control fish except the presence of *Micrococcus* in the mid gut of fish exposed to 30.5% effluent. It could be noted that the percent composition of the bacterial genera had changed in
Table 8.3 Generic Composition of the bacterial strains isolated from the stomach, mid gut and hind gut of control and treated *C. striatus*.

<table>
<thead>
<tr>
<th>Effluent Concentration</th>
<th>Source</th>
<th>Bacillus</th>
<th>Micrococcus</th>
<th>Pseudomonas</th>
<th>Achromobacter</th>
<th>Flavobacterium</th>
<th>No of isolates tested</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 %</td>
<td>Stomach</td>
<td>5 (20)</td>
<td>2 (8)</td>
<td>11 (44)</td>
<td>3 (12)</td>
<td>4 (16)</td>
<td>25</td>
</tr>
<tr>
<td></td>
<td>Mid gut</td>
<td>7 (25)</td>
<td>-</td>
<td>11 (39)</td>
<td>10 (36)</td>
<td>-</td>
<td>28</td>
</tr>
<tr>
<td></td>
<td>Hind gut</td>
<td>9 (30)</td>
<td>-</td>
<td>14 (47)</td>
<td>7 (23)</td>
<td>-</td>
<td>30</td>
</tr>
<tr>
<td>30.5 %</td>
<td>Stomach</td>
<td>4 (20)</td>
<td>2 (10)</td>
<td>7 (35)</td>
<td>5 (25)</td>
<td>2 (10)</td>
<td>20</td>
</tr>
<tr>
<td></td>
<td>Mid gut</td>
<td>5 (20)</td>
<td>1 (4)</td>
<td>11 (44)</td>
<td>8 (32)</td>
<td>-</td>
<td>25</td>
</tr>
<tr>
<td></td>
<td>Hind gut</td>
<td>7 (33.3)</td>
<td>-</td>
<td>10 (48)</td>
<td>4 (19)</td>
<td>-</td>
<td>21</td>
</tr>
<tr>
<td>61 %</td>
<td>Stomach</td>
<td>4 (18.2)</td>
<td>-</td>
<td>11 (50)</td>
<td>7 (32)</td>
<td>-</td>
<td>22</td>
</tr>
<tr>
<td></td>
<td>Mid gut</td>
<td>6 (23.1)</td>
<td>-</td>
<td>13 (50)</td>
<td>7 (30)</td>
<td>-</td>
<td>26</td>
</tr>
<tr>
<td></td>
<td>Hind gut</td>
<td>7 (25)</td>
<td>-</td>
<td>15 (54)</td>
<td>6 (21)</td>
<td>-</td>
<td>28</td>
</tr>
</tbody>
</table>

Values in the parenthesis indicate the percentage composition.
Table 8.4  Distribution of physiological grouping of various bacterial strains isolated in the stomach, mid gut, hind gut, of control and treated *C. striatus.*

<table>
<thead>
<tr>
<th>Effluent Concentration</th>
<th>SOURCE</th>
<th>Physiological Grouping</th>
<th>No of isolates tested</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Amylolytic</td>
<td>Gelatinolytic</td>
</tr>
<tr>
<td>0 %</td>
<td>Stomach</td>
<td>7 (28)</td>
<td>11 (44)</td>
</tr>
<tr>
<td></td>
<td>Mid gut</td>
<td>9 (32)</td>
<td>13 (46)</td>
</tr>
<tr>
<td></td>
<td>Hind gut</td>
<td>11 (37)</td>
<td>17 (57)</td>
</tr>
<tr>
<td>31.5 %</td>
<td>Stomach</td>
<td>9 (45)</td>
<td>5 (25)</td>
</tr>
<tr>
<td></td>
<td>Mid gut</td>
<td>11 (44)</td>
<td>7 (28)</td>
</tr>
<tr>
<td></td>
<td>Hind gut</td>
<td>10 (48)</td>
<td>5 (24)</td>
</tr>
<tr>
<td>61 %</td>
<td>Stomach</td>
<td>8 (36)</td>
<td>4 (18)</td>
</tr>
<tr>
<td></td>
<td>Mid gut</td>
<td>11 (42)</td>
<td>6 (23)</td>
</tr>
<tr>
<td></td>
<td>Hind gut</td>
<td>13 (46)</td>
<td>7 (25)</td>
</tr>
</tbody>
</table>

Values in parenthesis indicate the percentage composition.
30.5% concentration. There was a decline in the percentage of *Achromobacter* and *Flavobacterium* and marginal increase in the percentage of *Bacillus, Micrococcus* and *Pseudomonas* when compared with control fish. *Pseudomonas, Achromobacter* and *Bacillus* formed the major bacterial flora in the fish exposed to 30.5% effluent.

*C. striatus* exposed to 61% effluent showed higher percentage on the occurrence of *Pseudomonas* the microflora. A slight decrease in the percentage of *Bacillus* and *Achromobacter* was also noticed. But the genera *Micrococcus* and *Flavobacterium* were totally disappeared in all the tissues which were present in the control.

### 8.3.4 Physiological grouping of bacteria

The physiological grouping (Amylolytic, Gelatinolytic, Caseinolytic and Lipolytic) of the bacterial strains isolated from the stomach, mid gut and hind gut of the control and exposed *C. striatus* is represented in Table 8.4. In the control samples the dominance of caseinolytic (55%) and gelatinolytic (49%) strains were observed followed by amylolytic (32%) and lipolytic (20%) bacteria respectively. In general, hind gut of control fish harboured the maximum percentage of all physiological groups tested followed by mid gut and stomach. A significant change was evident in the distribution of the four physiological groups in the effluent treated fish (30.5%) and the strains differed widely in their occurrence in the regions studied (Table 8.4). Amylolytic and lipolytic bacteria showed an increase in percentage in all the digestive parts. Considerable reduction in the percentage of gelatinolytic and caseinolytic strains was observed in the fish treated with 30.5% effluent. There was a phenomenal fall in percentage of gelatinolytic and caseinolytic bacteria and raise in amylolytic and lipolytic bacteria in all the digestive parts tested. The occurrence of the physiological groups of bacterial strains in the fish exposed to 61% concentration showed an increase in amylolytic strain but a decrease in 30.5% effluent treated fish. The gelatinolytic and caseinolytic strains showed further decline in the fish exposed to 30.5%; while there was a fluctuational increase of lipolytic strains in entire digestive system. The hind gut of the treated fish exposed to 61% effluent showed an increase in the percentage of all physiological groups of strains.
8.4 Discussion

The present investigation concerned with the gut digestive tract microbiota of the control and effluent treated *C. striatus* and highlights the questions and process that merit attention in an comprehensive understanding of the role of digestive system; microbes in the physiology of host. Enzymes of the digestive system have been studied in detail in several invertebrates and vertebrates by a number of researchers. Graham (1932) initiated such a study of digestive enzymes in a gastropod and formulated a method for the study of carbohydrases. Since then, enzyme studies had been undertaken to include proteases and lipase as well. The study of digestive enzymes throw light on the capabilities of an organism to tackle different types of food stuffs, the plethora of enzymes that orchestrate the digestive process, the relative efficiency of the digestive system and the overall efficiency of these digestive system as a whole. Ferreri (1958), studied extensively the digestive enzymes of the gastropod *Helix pomatia*. Galii and Giese (1959) analysed in detail the digestive enzymes of herbivorous gastropod *Tegula funebralis*. Owen (1966) recorded the digestive enzymes and their role in carnivorous genera of gastropods.

The present study indicated that *C. striatus* was moderately efficient in digesting the various types of food materials. The hind gut (just a passage for way out of digested food) was known for poor digestive capability. On the other hand, the stomach and the mid gut were capable of digesting the food stuff with the help of their battery of enzymes; between the two, the midgut was more efficient as shown by the rate of the enzyme activity in the control. The present investigation revealed the presence of ploysaccharase, glycogenase in the midgut and their absence in the stomach and hind gut. Protease activity was very high in the hind gut. A remarkable feature was the complete absence of cellulase and maltase in the entire digestive system of *C. striatus*. This showed that the fish was incapable of utilising the cellulose and maltase poorly present in its carnivorous diet. Amylase is the only polysaccharase available in the fish in considerable quantities. *C. striatus* was efficient as far as disaccharide digestion is concerned and it showed high digestion of lipids. Lipase activity was noticed in stomach, mid gut and hind gut of *C. striatus*.

Effluent treatment slightly interfered with the activity of the digestive enzymes. There was a moderate reduction in the rate of enzyme activity and some enzymes appear to be absent as evident from lack of enzyme activity. The impairment of enzyme action was a general rule in the treated fish and was applicable to all the enzymes. By virtue of the beneficial role played by the gut microflora in the digestion of food stuffs by the host organism, several researchers studied the microbiology of the digestive system of invertebrates and vertebrates. Liston (1955) and Shewan (1961) reported the occurrence of intestinal bacterial flora and their role in performing the function of degrading detrital material for nutrition, leaving the role of digestion to the native enzymes of the host. Karthiayani and Iyer (1967) reported a heavy bacterial load in the gut of oil sardine. Simudu and Asio (1968) reported a similar occurrence of bacterial population in the gut of Japanese flat fish. The harbouring of bacterial flora in the digestive tract of marine fish was reported by Sera and Ishida (1972). Earlier Krishnamoorthy *et al.*, (1965) studied the role of microbes in the nutrition of some estuarine and marine bivalves. The involvement of gut microflora in the digestive process of the host has been recorded by Venkateswaran (1981); Sudha (1981); Palaniappan (1982); Kanakasabai (1985); Vanajakumar (1980); Devendran *et al.*, (1986) and Maya and Nair (1988).
Lessel (1989) studied the effect of temperature on the bacterial flora in the gut of rainbow trout and African cat fish. The role of microflora in synthesising organic molecules within the gut of Olive fruit fly was reported by Tsiropoulos and George (1989); these molecules became available to the host fly for its metabolism. Cellulolytic bacteria were characterised and identified up to generic level in the brown garden snail *Helix aspera* by Lesel et al., (1989). Sakata (1989) gave a complete list of microflora present in the digestive tract of fish and shell fish and the beneficial role of the symbionts in digestion. He stated that tetrotoxin present in the buffer fish was produced originally by the intestinal bacteria and the toxin was incorporated in the surface layers of the digestive tract to be accumulated in certain organs of the fish. Onarhein and Raa (1989) demonstrated that the bacteria belonging to family *Vibrionaceae* live in close association with the intestinal wall of marine fish such as herring, cod and salmon. Morairty (1989) gave an account on the ingestion and digestion of microorganisms by aquatic animals and the function of the gut flora in providing nutrients to the host. Using isotope labelled food pellets Bianchi et al., (1989) showed that 70-85% of the nitrogen assimilated by prawns came via the heterotrophic microbial communities. Sarkar (1989) reported that the gut of termites harboured several *Coccoid of Coccobacillus* type of bacteria which played a role in cellulolytic activity. Strom and Olafsen (1989) opined that some *Vibrio* species were found among the indigenous microflora of wild-captured juvenile cod and they were non pathogenic.

In the present study the microbes inhabiting the digestive tract of *C. striatus* have been investigated in the control and effluent treated (30.5 and 61%) fish. The population density of heterotrophic bacteria in each of the three chosen regions varied significantly. In the control, the hind gut lodged a higher number of bacteria followed by the midgut. Stomach contains a less bacterial density on account of being a short passage where food does remain too long. The stomach was the region where the various digestive enzymes help the digestive activity. The bacteriolytic action of the digestive enzyme present in the stomach lysed certain bacterial types which are susceptible to digestive enzyme. This reason may be attributed to the occurrence of less bacterial numbers in the stomach of control fish (Karrupasamy, 1996 and Reena Banerjee, 1997). In the treated fish there was a remarkable decrease in the total viable bacteria. The pattern of distribution of microbes in the different region of digestive tract was slightly different. The hind gut exhibited the high bacterial numbers and the midgut a slightly smaller population.
In the treated fish (30.5% effluent), food intake was very much reduced and the passage of food along the tract was at a sluggish pace. The absence of substrates, due to low intake of food by the fish. Occurrence of higher bacterial number in the hind gut region may be explained that the bacteria of hind gut were well adopted with microenvironment of hind gut region. The stomach is the place where main digestive activity take place. The digestive enzyme lyses certain bacterial types which enters the stomach through the food. Those bacteria which can tolerate bacteriolytic activity of digestive enzymes of stomach can proliferate well in mid gut and hind gut regions. After exposure to high concentration (61%) there was a further reduction of the density of the bacterial population. When compared to control the bacterial density of the hind gut of the treated fish (61%) accounted 3% of the control bacterial population. The enhanced intake of food and the availability of substrates to the microbes resulted an increase in the bacterial density in all over the digestive tract of control fish. Karrupasamy (1996) and Reena Banerjee (1997) reported less number of bacteria in the stomach of fish *Esomus dantius* and *Oreochromis mossambicus* respectively.

The generic composition of the gut microflora of control fish consisted of *Bacillus*, *Micrococcus*, *Pseudomonas*, *Achromobacter* and *Flavobacterium*. The *Achromobacter* and *Pseudomonas* genera were found dominant followed by *Bacillus*. *Micrococcus* and *Flavobacterium* were the least represented population and were present only in the stomach. Szabo (1989) was of the view that the "gut coryne-forms" represent a large heterogeneous complex of very different genera and species of nocardioform and coryneform bacteria. *Pseudomonas* species are mostly pathogens, the *Pseudomonas* occurring in *C. striatus* is non pathogenic to fish as stated by Onarheim and Raa (1989).

According to Sakata (1989), the genus *Aeromonas* and *Pseudomonas* are predominant in the intestinal microflora of the freshwater salmonids. The present work confirms the occurrence of *Pseudomonas* in the freshwater fish *C. striatus*. The gut of the effluent exposed (30.5%) fish showed the dominance of *Pseudomonas*, *Achromobacter* and *Bacillus*. The genus *Micrococcus* represented in mid gut sample. *Flavobacterium* was found absent in the midgut and hind gut. An analysis of the generic composition of the bacterial strains isolated from the fish exposed to 61% concentration showed a slight reduction in number from 30.5% concentration and an increase...
from the control. *Micrococcus* and *Flavobacterium* were eliminated in this concentration (61%) in all the digestive parts. The absence of these forms may be due to the susceptibility to the toxicity of effluent and for the non availability of specific substrates or nutrients in the food of the fish exposed to higher concentration.

With regard to the distribution of the physiological groups within the digestive system of the control fish, the hind gut appeared to be the most favoured habitat for all the physiological groups of (amylolytic, gelatinolytic, caseinolytic and lipolytic) bacteria; the proteolytic forms (gelatinolytic and caseinolytic) constitute the majority. The amylolytic strains ranked third and lipolytic bacteria showed the least. The hind gut lodged the highest population of all physiological groups of strains determined. Stomach is occupied by the smallest percentage of microbial population of each physiological group in control. In the treated fish (30.5%) in all the digestive regions studied, the amylolytic and lipolytic populations increased when compared to the control. The gelatinolytic and caseinolytic forms registered a fall in their percentage.

The drastic reduction of caseinolytic population in the hind gut of 30.5% effluent exposed fish was a remarkable feature. The enhanced amylolytic and lipolytic activity of the bacteria may be attributed to the availability of moderate amounts of polysaccharides which were not digested by the enzymes of microbial origin of the fish as evident from the low amylase activity. The low percentage of physiological groups in general may be attributable to the scarcity of substrates for the bacteria. Fish exposed to high concentration (61%) showed, a further decrease of all the physiological strains. This reduction in the density was perhaps due to the high concentration of effluent. The proportionate representation of four physiological groups was not similar to control.

**Correlation between microflora and native enzymes**

There was a complementation of the native enzymes by bacterial activity of the gut microflora in digesting the food in the gastropod *Bursa spinosa* (Muthu, 1989). He showed that the abundance of the physiological groups of bacteria and the efficiency of the native enzymes in each of the organs of the digestive system were inversely proportional in *B. spinosa*. The decrease in digestive capability of the native enzymes of the snail was amply compensated by
the gut microflora and thus maximal utilization of the nutrients in the food was attained. In the light of this work it may be stated that in *C. striatus* also there was appreciable complementary activity of the bacteria with the digestive enzymes of *C. striatus* if any part does not secrete an enzyme on its own the bacteria become complementary in function and help in the digestion of food. The *C. striatus* is having carnivorous mode of nutrition. The incidence of higher percentage of proteolytic bacterial (Gelatinolytic and Caseinolytic) in the control fish revealed that the microbial protease may help in the normal digestive activity of the animal. It can be safely assumed that the gut microflora have a significant role in the digestion of *C. striatus*. 