Studies on L-asparaginase enzyme of actinomycetes isolated from estuarine fishes

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Abstract: Actinomycetes were isolated from different organs viz. skin, gills and gut contents of three species of fishes viz. Mugil cephalus (Linnaeus, 1758), Chanos chanos (Forskal, 1775) and Etroplus suratensis (Bloch, 1780) using three different media from the Vellar estuary, situated along the southeast coast of India. Among the three fishes, M. cephalus harboured highest number of actinomycetes population in all the three body parts examined followed by C. chanos and E. suratensis. Out of the three body parts of all fishes, gut contents had highest actinobacteria population followed by gills and skin. Among the three media used for isolation of actinomycetes, Kuster’s agar medium was found to be suitable than the starch casein agar and glucose asparagine agar media. Out of the 40 strains isolated, only six strains (LA-2, LA-8, LA-15, LA-20, LA-29 and LA-35) showed significant L-asparaginase activity and were taken up for further studies. Impact of various physical and chemical factors such as pH, temperature, sodium chloride concentration, carbon sources and amino acids on the growth of actinomycetes and L-asparaginase activity was also studied. Optimum growth and enzyme activity was noticed under pH 7 to 8, temperature 37°C, 1-2% sodium chloride concentration, succrose as carbon source and without any amino acids. Analysis of the cell components of the isolated strains has revealed the wall type - I (the wall type - I is typical for the genus Streptomyces) and the strains were micromorphologically similar to the genus Streptomyces. Hence, the morphological, physiological and biochemical along with the micromorphological results of the L-asparaginase producing strains were compared and the strains were tentatively identified as Streptomyces aureofaciens (LA-2), S. chattanoogenesis (LA-8), S. hawaiiensis (LA-15), S. orientalis (LA-20), S. canus (LA-29) and S. olivoviridis (LA-35).

Key words: Estuarine fishes, Actinomycetes, L-asparaginase enzyme, pH, Temperature, Sodium chloride, Amino acids, Carbon compounds

Introduction

The enzyme L-asparaginase (L-asparagine amidohydrolase, E. C. 3.5.1.1) has attracted much attention in the past decades because of its antineoplastic activity (Crowther, 1971; Mc Creadie et al., 1973; Wriston and Yellin, 1973). This enzyme is used in the treatment of acute lymphatic leukemia with 60-80% remission (Claussen, 1986; Yoshimoto, 1986). Normal cells can synthesise their own L-asparaginase with the help of an enzyme known as L-asparagine synthetase, whereas the malignant cells deprived of this enzyme requires an exogenous source of L-asparagine for their growth and multiplication (Selvakumar et al., 1991). The L-asparaginase splits the L-asparagine, an important nutrient for cancer cells for the protein synthesis to make new cancer cells, into L-aspartic acid and ammonia (Selvakumar et al., 1991). Administration of L-asparaginase depletes exogenous L-asparagine and causes the death of malignant cells.

Occurrence of this enzyme has been reported in bacteria, actinomycetes and fungi (Balakrishnan Nair et al., 1977; Maya et al., 1992; Mathew et al., 1994). Though actinomycetes are ubiquitous in nature including water and sediments of the estuarine environment, the actinomycetes strains present in the living organisms including fishes shows good enzymatic activity than the strains isolated from the water and sediment samples (Dhevendran and Annie, 1999). Likewise actinomycetes are providing good L-asparaginase enzyme when compared to bacterial and fungal sources. Moreover, information on the occurrence of the L-asparaginase enzyme in actinomycetes associated with estuarine fishes of the southeast coast of India is lacking. Hence the present investigation aims to study the L-asparaginase activity of actinomycetes associated with different parts of three species of estuarine fishes viz. Mugil cephalus, Chanos chanos and Etroplus suratensis under different culture conditions like different pH, temperature, sodium chloride, carbon sources and amino acids and also to identify the potential L-asparaginase producing actinomycetes through chemotaxonomical and conventional methods of identification.

Materials and Methods

Isolation of actinomycetes: Fish samples Mugil cephalus, Chanos chanos and Etroplus suratensis were collected using cast net from the Vellar estuary. Samples were kept in sterile polyethylene bags and transported to the laboratory under ice for microbiological analysis. A small portion of the gills and the skin were removed aseptically with the help of forceps and scissors from these fishes. Before the alimentary tract of the fish was removed, the body surface was wiped with 70% ethanol by using sterile cotton (Sudha et al., 2002). The abdomen was
opened aseptically and then the alimentary tract was carefully taken out. The contents of the alimentary tract were squeezed out with forceps. Then, one gram of the squeezed out gut contents, gills and skin samples were taken and homogenized separately in a sterile mortar and pestle. The samples were serially diluted with filtered and sterilized 50% seawater. One ml of the serially diluted samples were plated (triplicate) in petriplates containing Kuster's agar (KUA), glucose asparagine agar (GAA) and starch casein agar (SCA) media and incubated at 35°C for seven days. The leathery colonies of actinomycetes that appeared on the petriplates were counted from the 5th day onwards up to 28th day. All the colonies that grew on the petriplates were sub cultured and were maintained in slants.

**Assay for L-asparaginase activity:** Actinomycetes from different parts of fishes were screened for L-asparaginase activity. The packed cells (5mg/ml) were suspended in distilled water and this was inoculated into 5 ml of glycerol-asparagine broth and incubated for seven days at 37°C temperature. After the 7th day, the broth was filtered through whatman number 1 filter paper and the paper was kept in incubator at 37°C for five days and the difference in weight was expressed as growth of actinomycetes in terms of dry weight. Activity of L-asparaginase was measured by adding 0.5 ml of Nessler's reagent to the filtered cultured broth (Dhevendran and Annie, 1999). Within five minutes, a yellow colour was developed. Then the sample was centrifuged and absorbance of the supernatant was read using a UV visible spectrophotometer (Hitachi) at a wave length of 450 nm (Wriston, 1971). The ammonia content was estimated using standard ammonium chloride solution and protein content of the enzyme preparation was estimated by following the method of Lowry et al. (1951). L-asparaginase activity is expressed in International units (IU)/mg of protein. Ammonium sulphate was used as standard.

**Effect of pH, temperature, sodium chloride, carbon compounds and amino acids on L-asparaginase activity and growth of the actinomycetes:** L-asparaginase activity and growth of the actinomycetes were measured at different pH, temperature, sodium chloride concentrations, carbon compounds and amino acids. The experiments were conducted in 250ml Erlenmeyer flasks containing the sterilized (at 15 lbs pressure for 15 minutes) glycerol asparagine broth. The flasks were cooled and the strain was inoculated and incubated for different parameters as described below.

**Effect of pH:** This was studied by varying the pH of the glycerol asparagine broth by addition of buffer solution ranging from pH 6 to 10. After inoculation of the strain, it was incubated for seven days at 37°C temperature.

**Effect of temperature:** After inoculation of the strain in glycerol asparagine broth, it was incubated at various temperatures such as 25°C, 29°C, 33°C, 37°C, 41°C and 45°C for seven days.

**Effect of sodium chloride concentrations:** To study the tolerance of strains towards sodium chloride concentrations, the glycerol asparagine broth prepared with distilled water was incorporated with sodium chloride at varying concentrations such as 0.05%, 0.1%, 0.5%, 1%, 2%, 3% and 4%. After inoculation of the strain, it was incubated at 37°C for seven days.

**Effect of various carbon compounds:** The glycerol asparagine broth was used for studying the effect of various compounds such as glucose, sucrose, lactose, raffinose and mannitol. The broth was distributed into various flasks and 1% of each carbon source was then added before inoculation of the strain and it was incubated for seven days at 37°C temperature.

**Effect of various amino acids:** The glycerol asparagine broth was used for studying the influence of organic matter such as methionine, tryptophan, L-glutamic acid and threonine. The broth was distributed into various flasks and 0.8 ml of each amino acid was then added and incubated for seven days at 37°C temperature.

At the end of the incubation period, the cells were harvested and dried at 35°C temperature in the incubator. Weight of the cells was taken and expressed as g. dry weight (Dhevendran and Annie, 1999). L-asparaginase activity was determined by the procedure as described earlier.

**Taxonomic investigation:** The genus level identification was made for the six strains which showed good enzymatic activity using cell wall composition analysis and micromorphological studies (Lechevalier and Lechevalier, 1970). Characterization of L-asparaginase producing actinomycetes was made by following the methods described by Shirling and Gottlieb (1996) using the standard culture medium yeast extract-malt extract agar (ISP medium 2). The species level identification of these strains was made based on the keys of Nonamura (1974).

**Results and Discussion**

**Actinomycetes population density:** Actinomycetes population density varied in the different parts viz. skin, gills and the gut contents of all the three species of fishes and also in different media used.

In the **KUA medium**: Population density of actinomycetes recorded from the skin of all the three species of fishes varied from 0.37 to 0.65 x 10⁷ CFU/g with the minimum (0.37 x 10⁷ CFU/g) in *E. suratensis* and the maximum (0.65 x 10⁷ CFU/g) in *M. cephalus*. In the case of the gills, it varied from 0.98 to 1.35 x 10⁷ CFU/g with the minimum (0.98 x 10⁷ CFU/g) in *E. suratensis* and the maximum (1.35 x 10⁷ CFU/g) in *M. cephalus*. In the case of the gut contents, the maximum was observed (4.15 x 10⁶ CFU/g) in *M. cephalus* and the minimum (3.15 x 10⁵ CFU/g), in *E. suratensis* (Fig. 1).
In the GAA medium: Actinomycetes population density recorded from the skin of all the three species of fishes varied from 0.25 to $0.32 \times 10^2$ CFU/g. The highest density ($0.32 \times 10^2$ CFU/g) was observed in *M. cephalus*, followed by *C. chanos* ($0.31 \times 10^2$ CFU/g) and *E. suratensis* ($0.25 \times 10^2$ CFU/g). In the case of the gills, population density ranged from $0.85 \times 10^2$ to $1.12 \times 10^2$ CFU/g. The highest density ($1.12 \times 10^2$ CFU/g) was recorded in *M. cephalus*. This was followed by *C. chanos* ($1.01 \times 10^2$ CFU/g) and *E. suratensis* ($0.85 \times 10^2$ CFU/g). While in the case of gut contents, population density fluctuated between $2.98$ and $3.35 \times 10^2$ CFU/g with the maximum ($3.35 \times 10^2$ CFU/g) in *M. cephalus* followed by *C. chanos* ($3.01 \times 10^2$ CFU/g) and *E. suratensis* ($2.98 \times 10^2$ CFU/g) (Fig. 2).

In the SCA medium: The mean population density of actinomycetes recorded from the skin of all the three species of fishes varied from $0.19$ to $0.29 \times 10^2$ CFU/g with the minimum ($0.19 \times 10^2$ CFU/g) in *E. suratensis* and the maximum ($0.29 \times 10^2$ CFU/g) in *M. cephalus*. In the case of the gills, it varied from $0.27$ to $0.49 \times 10^2$ CFU/g with the minimum ($0.27 \times 10^2$ CFU/g) in *E. suratensis* and the maximum ($0.49 \times 10^2$ CFU/g) in *M. cephalus*. In the case of the gut contents, the maximum was observed ($1.45 \times 10^2$ CFU/g) in *M. cephalus* and the minimum ($1.01 \times 10^2$ CFU/g), in *E. suratensis* (Fig. 3).

Actinomycetes populations varied broadly in their density in the different body parts of all three species of fishes and also in different media used. Gut contents harboured more population density compared to other parts of the fishes. The reason for this could be that the gills and the gut contents are exposed to the entry of variety of microbial populations including actinomycetes along with diverse food particles and more numbers of microbes could have been retained in the guts than the gills as the latter are continuously washed off with large volume of water. Such occurrence of actinomycetes could be beneficial to the fishes either in the production of (microbial) enzymes useful for the
digestion or in the secretion of growth factors and vitamins (by microbes) which are useful for fishes. Higher percentage of occurrence of actinomycetes in the gut contents of all the three fish species up to 3.15 x 10^2 CFU/g, as observed in the present study, could be also due to the production of mycoid slime in the guts which can act as nutrient source for actinomycetes. This would help in symbiotic or commensal relationship between the host and actinomycetes. In a recent study from the Veli lake of Kerala state, this relationship has been emphasized (Dhevendaran and Annie, 1999).

There were marked variations in the number of colonies of actinomycetes in the three media used (Fig. 4). Maximum number of colonies was enumerated in KUA medium followed by GAA medium and SCA medium. Reported that the KUA medium is best suited for the isolation of actinomycetes from the water, sediment, seaweed and molluscs samples of the Vellar estuary. The present study confirms that the same medium can also be used for isolation of actinomycetes from the fish samples.

**L-asparaginase activity:** As it is very difficult to obtain sufficient quantities of L-asparaginase from marine microorganisms, not many studies on this enzyme have been carried out. Except for the report of Mathew et al. (1994) and Koshy et al. (1997) on L-asparaginase from antagonistic Streptomyces sp. isolated from the Villorita cyprinoids and different samples of the Veli lake, Kerala, no literature is available from Indian coastal regions. In the present study, out of 40 actinomycetes strains isolated from the fishes and tested, only 6 showed good L-asparaginase activity.

Table 1 shows the L-asparaginase activity and growth of 6 strains of actinomycetes, which exhibited good L-asparaginase activity. All the cultures exhibited activity and growth to different degrees. The strain LA-29 exhibited maximum enzymatic activity (35.6 μg ammonia/ml/h) followed by the strain LA-15 (34.5 μg ammonia/ml/h).

<table>
<thead>
<tr>
<th>Strain no.</th>
<th>Activity (μg ammonia/ml/h)</th>
<th>Growth (g. dry weight)</th>
</tr>
</thead>
<tbody>
<tr>
<td>LA – 2</td>
<td>33.9</td>
<td>0.12</td>
</tr>
<tr>
<td>LA – 8</td>
<td>32</td>
<td>0.03</td>
</tr>
<tr>
<td>LA – 15</td>
<td>34.5</td>
<td>0.10</td>
</tr>
<tr>
<td>LA – 20</td>
<td>31.2</td>
<td>0.04</td>
</tr>
<tr>
<td>LA – 29</td>
<td>35.6</td>
<td>0.09</td>
</tr>
<tr>
<td>LA – 35</td>
<td>33.1</td>
<td>0.08</td>
</tr>
</tbody>
</table>
ammonia/ml/h) and the strain LA-2 (33.9 µg ammonia/ml/hr). Mathew et al. (1994) reported the L-asparaginase activity in Streptomyces sp. isolated from the foregut and hindgut of the clam, V. cyprinoides. Furthermore, Koshy et al. (1997) have also noticed more-or-less similar pattern of enzymatic activity in Streptomyces sp. isolated from the sediments, fishes and molluscs of Veli lake, Kerala. They have also reported that the occurrence of Streptomyces in fish and molluscs and synthesis of L-asparaginase enzyme could be due to the production of natural substrate by the host organisms that can be ideally used by Streptomyces. This could be the reason for the production of L-asparaginase by the isolated strains from the fishes. The strain LA-29 produced more L-asparaginase (35.6 µg ammonia/ml/h) with a growth of 0.09 g. in terms of dry weight followed by the strain LA-15, which produced 34.5 µg ammonia/ml/h of L-asparaginase in the 0.10 g. of dry weight. However, the strain LA-8 and strain LA-20 exhibited better enzymatic activity when compared to their very low growth rate.

**Effect of pH, temperature, sodium chloride, carbon compounds and amino acids on L-asparaginase activity and growth of the actinomycetes:** Enzymatic activity was optimum at pH 7 and the growth was maximum at pH 8 (Fig. 5). This clearly indicates that the growth of actinomycetes and the enzyme activity are pH dependent. This observation is in conformity with the earlier findings of Maya et al. (1992), Mathew et al. (1994) and Koshey et al. (1997). In Penaeus indicus and Therapon jarbua, the enzyme activity and growth were also maximum at pH 7 (Dhevendran and Annie, 1999; Dhevendran and Anithakumari, 2002). The intracellular secretion of microbial cells or intracellular enzymes released after the rupture of cells could be the reason for the peak enzyme activity at pH 7.

Since the cultures were isolated from the aquatic organisms, study of the influence of temperature on the enzyme activity and growth of actinomycetes is imperative. It was observed that maximum activity and growth were at 37 °C for all the strains (Fig. 6), thereby, implying that they might have been transported from land sources as suggested by Okami (1984). Their adaptation to the aquatic environment was probably due to the metabolic changes and the production of L-asparaginase may

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**Fig. 5:** Effect of pH on L-asparaginase activity and growth of the strains

**Fig. 6:** Effect of temperature on L-asparaginase activity and growth of the strains
be unique (Dhevendaran and Anitha Kumari, 2002). It has already been reported by Maya et al. (1992) that optimum activity and maximum growth in Bacillus sp. and Moraxella sp. were at 37°C and Vibrio sp. exhibited maximum L-asparaginase activity at 68°C (Selvakumar et al., 1991) though it was isolated from the shellfish, Telescopium telescopium from the Vellar estuary. Recently, Dhevendaran and Annie (1999) and Dhevendaran and Anitha Kumari (2002) have observed maximum activity of this enzyme at 37°C in the mesophilic Streptomyces AQBS1 104 and AQBTJ 60, isolated from Penaeus indicus and Therapon jardae of the Veli lake, Kerala.

Since in the present study, actinomycetes were isolated from the estuarine fish, it was felt necessary to understand the influence of various concentrations of sodium chloride on its enzymatic activity and growth. The activity was recorded even at 0% and the optimum growth was observed at 1-2% sodium chloride concentration in all the strains (Fig. 7). Selvakumar et al. (1977), Maya et al. (1992), Mathew et al. (1994) and Dhevendaran and Anitha Kumari (2002) have also noticed similar pattern of enzyme activity in the marine sediments, marine bacteria, streptomycetes and Streptomyces respectively. The reduced enzyme activity at the higher concentration could be due to the formation of certain inhibitors in the growth medium or due to the inhibitory nature of increased sodium chloride level.

Among the different carbon sources used, the enzyme activity and growth were enhanced in sucrose, whereas lactose had an inhibitory effect (Fig. 8). Selvakumar et al. (1977) tested L-asparaginase activity in 24 carbon sources and found that lactose had inhibitory effect on the marine Vibrio sp. In Moraxella sp. and streptomycetes, the enzyme activity and growth were increased by the addition of sucrose and inhibited by lactose as observed by Maya et al. (1992) and Mathew et al. (1994).

Fig. 9 shows the effect of amino acids on L-asparaginase activity and growth of the isolated strains. Maximum activity of enzyme and growth of the strains were noticed without any amino acids. This observation is in conformity with the earlier findings of Mathew et al. (1994) and Dhevendaran Anitha Kumari et al. (2002) in Streptomyces sp. They observed higher L-asparaginase activity and growth of actinomycetes without any amino acids and thereby reported that the naturally available amino acids in the host organisms were sufficient for the maximum enzymatic activity and growth of actinomycetes.

### Table - 2: Morphological and micromorphological characteristics of six strains of L-asparaginase producing actinomycetes

<table>
<thead>
<tr>
<th>Strain no.</th>
<th>Species to which assigned</th>
<th>Aerial mass color</th>
<th>Melanoid pigment</th>
<th>Reverse side pigment</th>
<th>Soluble pigment</th>
<th>Spore chain</th>
<th>Spore surface</th>
</tr>
</thead>
<tbody>
<tr>
<td>LA-2</td>
<td>Streptomyces aureofasciulus</td>
<td>White</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>RF (Plate 1)</td>
<td>Smooth</td>
</tr>
<tr>
<td>LA-8</td>
<td>S. chattanoogenesis</td>
<td>White</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>Spiral</td>
<td>Spiny</td>
</tr>
<tr>
<td>LA-15</td>
<td>S. hawaiiensis</td>
<td>White</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>Spiral</td>
<td>Spiny</td>
</tr>
<tr>
<td>LA-20</td>
<td>S. orientalis</td>
<td>Yellow</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>RF (Plate 6)</td>
<td>Smooth</td>
</tr>
<tr>
<td>LA-29</td>
<td>S. canus</td>
<td>Grey</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>Spiral</td>
<td>Spiny</td>
</tr>
<tr>
<td>LA-35</td>
<td>S. olivoviridis</td>
<td>Grey</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>RF (Plate 9)</td>
<td>Spiny</td>
</tr>
</tbody>
</table>

RF-rectiflexible; + denotes presence; - denotes absence

### Table - 3: Utilization of carbon compounds by six strains of L-asparaginase producing actinomycetes

<table>
<thead>
<tr>
<th>Strain no.</th>
<th>Species to which assigned</th>
<th>A</th>
<th>X</th>
<th>I</th>
<th>M</th>
<th>F</th>
<th>R</th>
<th>S</th>
<th>R</th>
</tr>
</thead>
<tbody>
<tr>
<td>LA-2</td>
<td>Streptomyces aureofasciulus</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>±</td>
<td>+</td>
</tr>
<tr>
<td>LA-8</td>
<td>S. chattanoogenesis</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>LA-15</td>
<td>S. hawaiiensis</td>
<td>±</td>
<td>±</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>±</td>
<td>+</td>
</tr>
<tr>
<td>LA-20</td>
<td>S. orientalis</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>±</td>
<td>±</td>
</tr>
<tr>
<td>LA-29</td>
<td>S. canus</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>±</td>
<td>+</td>
</tr>
<tr>
<td>LA-35</td>
<td>S. olivoviridis</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

A- Arabinose, X-Xylose, I-Inositol, M-Manifold, F-Fructose, R-Rhamnose, S-Sucrose, R-Raffinose, + denotes presence, - denotes absence
± denotes doubtful
L-asparaginase enzyme of actinomycetes

**Fig. 7:** Effect of NaCl concentration on L-asparaginase activity and growth of the strains

**Fig. 8:** Effects of carbon compounds on L-asparaginase activity and growth of the strains

**Fig. 9:** Effects of amino acids on L-asparaginase activity and growth of the strains
Plate - 1: Strain LA 2 - Rectiflexibiles (RF) spores chain (400X)

Plate - 2: Strain LA 8 - Spiral (S) spore chain (400X)

Plate - 3: Strain LA 8 - Spores with spiny surface (15,000X)

Plate - 4: Strain LA 15 - Spiral (S) spore chain (400X)

Plate - 5: Strain LA 15 - Spores with spiny surface (15,000X)

Plate - 6: Strain LA 20-Rectiflexibiles (RF) spore chain (400X)

Plate - 7: Strain LA 29 - Spiral (S) spore chain (400X)

Plate - 8: Strain LA 29 - Spores with spiny surface (7,000X)

Plate - 9: Strain LA 35-Rectiflexibiles (RF) spore chain (400X)

Plate - 10: Strain LA 35 - Spores with spiny surface (4,500X)
**Taxonomic investigation:** Of the 40 strains tested for L-asparaginase activity, only six strains viz. LA-2, LA-8, LA-15, LA-20, LA-29 and LA-35 showed good activity (Table 1) and hence these strains were selected for identification.

All the six strains possess LL-Diaminopimelic acid and all the strains tested contain glycine in their cell wall. Presence of LL – Diaminopimelic acid along with glycine indicates the cell wall chemotype – I. The strains with chemotype – I do not have characteristic pattern of sugars (Lechevalier and Lechevalier, 1970).

The strains belonging to the wall type I are Streptomyces, Streptoverticillium, Chainia, Actinopycnidium, Actinosporangium, Elyttrasporangium, Microellobosporia, Sporichthyia and Intrasporangium (Lechevalier and Lechevalier, 1970). The micromorphological observations of the strains, LA-2, LA-8, LA-15, LA-20, LA-29 and LA-35 reveal that all these belong to the genus Streptomyces. The predominance of Streptomyces in any actinomycete population is a well known fact (Alexander, 1961).

The morphological, micromorphological, physiological and biochemical characteristics obtained for the L-asparaginase producing strains LA-2, LA-8, LA-15, LA-20, LA-29 and LA-35, tested in the present study are depicted in Table 2, 3. The results were compared with those of the Streptomyces species given in the key of Nonomura (1974) and those species described in the Bergey’s Manual of Determinative Bacteriology (Buchanan and Gibbons, 1974).

The result shows that except for the absence of production of melanoid and soluble pigments by the strain LA-2 (Table 2), all the other characters are exactly similar to those of S. aureofaciatus. Therefore, the strain LA-2 has been tentatively identified as S. aureofaciatus. In the strain LA-8, except for the difference in the utilization of inositol and production of soluble pigment, all the other properties are exactly similar to that of S. chattanoogenisis and hence this strain has been tentatively identified as S. chattanoogenisis (Tables 2, 3). The result further shows that except for the production of reverse side pigments and absence of utilization of mannitol by the strain LA-15 (Tables 2, 3), all the other characters are exactly similar to those of S. hawaiiensis. Therefore, the strain LA-15 has been tentatively identified as S. hawaiiensis. Table 3 shows that the strain LA-20 is weak in utilization of the carbon compounds viz. fructose. Except this, all the other properties are the same for LA-20 and S. orientalis. Therefore, the strain LA-20 has been identified as S. orientalis. The strain LA-29 differed from the reference strain S. canus, by not utilizing the carbon compound viz. rhamnose. However, LA-29 resembles the reference strain by showing close similarity in all the other characters and so the strain LA-29 has been identified as S. canus (Table 2). Similarly, the strain LA-35 is weak in the utilization of arabinose and production of soluble pigment. Except this, all the other characters of the strain LA-35 are exactly similar to that of S. olivoviridis and hence the strain LA-35 has been identified as S. olivoviridis (Tables 2, 3).

From the present study, it has been inferred that the finfishes viz. M. cephalus, C. chanos and E. suratensis of the Vellar estuaries are potential candidates for the isolation of L-asparaginase producing actinomycetes and the growth conditions have been optimized for culture of these strains and synthesis of L-asparaginase enzyme under laboratory conditions. Further, the indigenous constituents of the host organisms and the environmental factors have influence on the metabolic activity of microorganisms also. During the present study, six strains were identified from the different parts of fishes and assigned to different species of Streptomyces viz. Streptomyces aureofaciatus (LA-2), S. chattanoogenisis (LA-8), S. hawaiiensis (LA-15), S. orientalis (LA-20), S. canus (LA-29) and S. olivoviridis (LA-35), which possess good L-asparaginase enzyme activity. Further, studies are needed to develop novel anti cancer drugs of L-asparaginase enzyme extracted from actinomycetes.

As these fishes are available throughout the year in the estuaries in cheaper rates, hence, it is recommended to utilize the finfishes viz. M. cephalus, C. chanos and E. suratensis for the isolation of L-asparaginase producing actinomycetes in the commercial scale.

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