CHAPTER 5

FERMENTATION KINETICS OF L-ASPARAGINASE
PRODUCTION BY STRAIN EPD 27

5.1 INTRODUCTION

L-asparaginase therapy, alone is finding increased success in the management of acute lymphocytic leukemias. In recent years, complete remissions were obtained in more than 80% of patients treated with 3000 to 9000 IU/kg per day (Narta et al. 2007). Dosages of this magnitude require large amounts of purified enzyme and increasing recognition of successful therapy will generate increased demand. The production of L-asparaginase by using bacteria has attracted great attention owing to their cost effective and eco-friendly nature. The physicochemical conditions for production of L-asparaginase are found to be varied between organisms (Savitri et al. 2003). L-asparaginase is produced by large number of bacteria such as E. coli (Robison and Berk 1969), Erwinia aroideae (Liu and Zajic 1973), Serratia marcescens (Novak and Phillips 1974), Citrobacter (Bascomp et al. 1975), Vibrio succinogenes (Kafkewitz and Goodman 1974 and Radcliffe et al. 1979), Streptomyces (Mostafa and Salama 1979), Corynebacterium glutamicum (Mesas et al. 1990), Erwinia carotovora (Maladkar et al. 1993 and Aghaiypour et al. 2001), Pseudomonas stutzeri (Manna et al. 1995), Enterobacter aerogens (Mukherjee et al. 2000), Pseudomonas aeruginosa (Fattah and Olama 2002) Staphylococci (Prakhasam et al. 2007).
There has been much work reported on the effect of growth medium on enzyme production by members of the enterobacteria. Various carbon and nitrogen source were reported for the production of L-asparaginase by several different bacteria (Savitri et al 2003). L-asparaginase production is found to be improved by various medium modifications using different nitrogen sources (Prakhasam et al 2007). Cell growth and L-asparaginase production are usually stimulated by supplying the complex forms of nitrogen such as peptone and yeast extract and casein hydrolysate (Cedar and Schwartz 1968 and Kono and Asai 1969). Synthetic media modified with L-asparagine as a source of nitrogen was found to be stimulated more enzyme production than natural media (Mostafa and Salama 1979). Starch and L-asparaginase are found to be suitable sources for the production of L-asparaginase by Streptomyces (Mostafa 1982).

Thus far most publications on L-asparaginase production have dealt with the nutrient requirement of bacteria for optimal enzyme production. Far less research has been completed upon fermentation kinetics of enzyme production. Kinetic studies would allow the prediction of fermentation rate, product yield and the control of fermentation process. The study on the L-asparaginase activity of marine Streptomyces has been reported in detail (Dhevendaran and Annie 1999, Dhevagi and Poorani 2006 and Sahu et al 2007). Yet the study on the fermentation kinetics of the enzyme production has not been reported so far. In this present investigation, a marine Streptomyces sp strain EPD 27 producing novel antitumour L-asparaginase was discovered which is described in chapter 2, 3 and 4. Hence our interest turned towards the use of strain for the large scale production of the enzyme. In this chapter, the procedures used for the study of fermentation kinetics of L-asparaginase production by strain EPD 27 and the results obtained are discussed.
5.2 MATERIALS AND METHODS

The methodology of this chapter is organized as a series of study on the effect of various fermentation factors such as pH, temperature, aeration and NaCl are in Erlenmeyer flask. Further a series of study on the effect of L-asparagine on growth and production of L-asparaginase in a fermenter.

5.2.1 Effect of pH on the Production of L-asparaginase

The strain EPD 27 was inoculated into different 100 ml of L-asparagine (0.8 %) supplemented synthetic starch casein media in a 500 ml of Erlenmeyer flask. Each prepared in various pH such as 7 to 9 was incubated in a shaker cum incubator at a speed of 300 rpm at 30ºC for 120h. The growth of the mycelium was measured and expressed as dry weight (De Jong 1972). Yield of L-asparaginase was measured as described in earlier section 2.2.3.2.

5.2.2 Effect of Temperature on the Production of L-asparaginase

The strain EPD 27 was inoculated into different 100 ml L-asparagine (0.8%) supplemented synthetic starch casein medium (pH 8.5). It was incubated in a shaker cum incubator at various temperatures such as 25 ºC to 30 ºC at a speed of 300 rpm for 120 h. The growth of the mycelium was measured and expressed as dry weight (De Jong 1972). Yield of L-asparaginase was measured as described in earlier section 2.2.3.2.

5.2.3 Effect of Aeration on the Production of L-asparaginase

The strain EPD 27 was inoculated into various volumes such as 50, 100, 150, 200 and 250 ml of L-asparagine (0.8%) supplemented in synthetic starch casein medium in a 500 ml of Erlenmeyer flask and were incubated in a shaker cum incubator at a speed of 300 rpm at 30ºC for 5 days. The growth
of the mycelium was measured and expressed as dry weight (De Jong 1972). Yield of L-asparaginase was measured as described in earlier section 2.2.3.2.

**5.2.4 Effect of NaCl on the Production of L-asparaginase**

The strain EPD 27 was inoculated into different 100 ml of L-asparagine (0.8%) supplemented synthetic starch casein medium modified with different concentration of NaCl such as 1 to 5% in a 500 ml of Erlenmeyer flask and were incubated in a shaker cum incubator at a speed of 300 rpm at 30 ºC for 5 days (Dhevendaran and Annie 1999). The growth of the mycelium was measured and expressed as dry weight. Yield of L-asparaginase was measured as described in earlier section 2.2.3.2.

**5.2.5 Batch Fermentation**

The strain EPD 27 stock culture was maintained on starch casein agar slants containing starch 1 g, K₂HPO₄ 0.2 g, KNO₃ 0.2 g, NaCl 0.2 g, casein 0.03 g, MgSO₄·7H₂O 0.005 g, CaCO₃ 0.002 g, FeSO₄·7H₂O 0.001 g and agar 2 g at a temperature of 4 ºC. Regular sub culturing of the strain was carried out at an interval of every four weeks. Spore suspension was prepared from 5 day old culture grown on starch casein agar slant by adding 10 ml of sterile distilled water containing 0.01% of Tween 80 and the suspended spores were considered as inocula. Fermentations were carried out in a 3 L fermenter (Bioferm LS1-3L, Scigenics India Pvt. Ltd) with 1 L synthetic starch casein medium modified with different concentration of L-asparagine such as 0.4, 0.8, 1.2 and 1.6% (pH 8.5) were inoculated with 10 ml of spore suspension and incubated for 120 h. The pH was adjusted to 8.5 prior to sterilization. Temperature was maintained at 30º C. Aeration was at the rate of 1.0 L/min (0.1 vol of air per vol of medium per min), agitation rate was 300 rev/min which was provided by a magnetic stirrer. Samples (5 ml culture) were withdrawn at regular intervals of 24 h and were used for the determination of
growth rate and L-asparaginase assay. All fermentations were carried out separately and the growth of the mycelium were measured and expressed as dry weight. Yield of L-asparaginase was measured as described in earlier section 2.2.3.2.

5.3 RESULTS AND DISCUSSION

A series of experiments in Erlenmeyer flask was carried out to determine the effect of pH, temperature, aeration and agitation for the production of L-asparaginase by strain EPD 27. Followed by a series of fermentations in fermenter was carried out to study the effect of L-asparagine on growth rate and production of L-asparaginase by the optimized factors.

5.3.1 Effect of pH on the Growth Rate to Enzyme Synthesis

In general, the source of carbon used in fermentation medium mainly influence in pH changes. Glucose in the fermentation medium is found to inhibit the L-asparaginase production by lowering the pH which was responsible for the lower enzyme production in Serratia marcescens (Heineman and Howard 1970), E. coli (Barnes et al 1977) Proteus vulgaris (Tosa et al 1971) and Enterobacter aerogenes (Mukherjee et al 2000). Contrary observations have also been made on the enhancement of L-asparaginase yield by glucose in Serratia marcescens (Khan et al 1970) and in Staphylococcus sp 6A (Prakhasam et al 2007).

Various carbon and nitrogen sources were studied to control the acidic pH of the fermentation media, lactose and starch were found to be the best carbon source for the production of L-asparaginase by Erwinia aroideae at pH 8.5 (Liu and Zajic 1973). While analysing the previous study, starch was found to be the best carbon source for production of L-asparaginase by Streptomyces and starch casein medium was the suitable medium than the
natural media for the production of L-asparaginase (Mostafa and Salama 1979).

The pH and temperature optima for L-asparaginase production are the same as that for the growth of the enzyme producing organisms (Mesas et al 1990). The effect of pH on the production of L-asparaginase by strain EPD 27 was determined in different pH range. The optimum pH for the growth and enzyme yield was found to be pH 8.5 (Table 5.1). The previous studies have shown that the optimum pH for the growth and L-asparaginase activity was found to be pH 7 (Dhevendaran and Annie 1999). They also suggest that marine Streptomyces are found to have similar pH optima for the growth and L-asparaginase activity. The results obtained in this study for the effect of pH are found to be good agreement with findings of Mesas et al (1990).

Table 5.1 Effect of pH on the growth and L-asparaginase production

<table>
<thead>
<tr>
<th>pH</th>
<th>Dry weight of mycelium (mg/ml)</th>
<th>L-asparaginase (IU/mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>7.0</td>
<td>0.87</td>
<td>0.04</td>
</tr>
<tr>
<td>7.5</td>
<td>0.92</td>
<td>0.12</td>
</tr>
<tr>
<td>8.0</td>
<td>1.07</td>
<td>0.72</td>
</tr>
<tr>
<td>8.5</td>
<td>2.79</td>
<td>1.92</td>
</tr>
<tr>
<td>9.0</td>
<td>2.08</td>
<td>1.63</td>
</tr>
<tr>
<td>9.5</td>
<td>1.62</td>
<td>1.47</td>
</tr>
<tr>
<td>10.0</td>
<td>1.20</td>
<td>1.10</td>
</tr>
</tbody>
</table>

There was a lesser growth and enzyme yield in pH below 8 when comparing to the pH above 9.0. This may be due to the fact that the higher pH increases the growth and enzyme production due to the production of high ammonia. This ammonia produced in the medium by the action of L-asparaginase is found to support the enzyme synthesis which indicates the absence of nitrogen catabolite repression. This stimulatory effect of ammonia
for the L-asparaginase production has been reported in Vibrio succinogenes (Albanese and Kafkewitz 1978). On contradiction, inhibitory effect of the ammonia on the production of L-asparaginase has been shown in Proteus vulgaris (Tosa et al 1971) and Bacillus subtilis (Sun and Setlow 1991).

5.3.2 Effect of Temperature on the Growth Rate to Enzyme Synthesis

The effect of temperature on the growth and L-asparaginase production was studied in different temperatures. The optimum temperature for the growth and L-asparaginase production was found to be 30 °C (Table 5.2). Similar temperature optima for the growth and production of L-asparaginase by Streptomyces were reported by Mostafa and Salama (1979). On contradiction, Dhevendaran and Annie (1999) observed that temperature optima for growth and L-asparaginase production were found to be 35 °C for marine Streptomyces.

Table 5.2 Effect of temperature on the growth and L-asparaginase production

<table>
<thead>
<tr>
<th>Temperature (°C)</th>
<th>Dry weight of mycelium (mg/ml)</th>
<th>L-asparaginase (IU/mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>25</td>
<td>0.30</td>
<td>0.21</td>
</tr>
<tr>
<td>26</td>
<td>0.38</td>
<td>0.22</td>
</tr>
<tr>
<td>27</td>
<td>0.42</td>
<td>0.22</td>
</tr>
<tr>
<td>28</td>
<td>0.57</td>
<td>0.53</td>
</tr>
<tr>
<td>29</td>
<td>1.38</td>
<td>0.57</td>
</tr>
<tr>
<td>30</td>
<td>1.42</td>
<td>0.94</td>
</tr>
<tr>
<td>31</td>
<td>0.90</td>
<td>0.63</td>
</tr>
<tr>
<td>32</td>
<td>0.35</td>
<td>0.37</td>
</tr>
<tr>
<td>33</td>
<td>0.20</td>
<td>0.31</td>
</tr>
</tbody>
</table>
While observing the temperature at 29 °C the growth rate was similar to that of 30 °C but the enzyme production was found to be less. Further, all the subsequent studies were carried out at pH 8.5 and 30 °C.

5.3.3 Effect of Aeration on the Growth Rate to Enzyme Synthesis

Gentle aeration enables to obtain both good growth and high L-asparaginase yield (Bilimoria 1969). Serratia marcescens produces large amount of L-asparaginase with limited aeration than it does anaerobically (Barnes et al 1977). Most of the organisms demand yeast extract for the growth and L-asparaginase production (Liu and Zajic 1973). While using yeast extract, it was found to increase the viscosity of the medium and thereby reducing the oxygen uptake, good mixing was critical especially when fermentation medium becomes viscous (Mukherjee et al 2000). In such cases, growth was limited by transfer of oxygen to the cell surface rather than by oxygen solubility which was observed in E. coli and E. chrysanthemi (Prakhasam et al 2007).

The effect of aeration was studied in various volumes of medium in Erlenmeyer flask. The flask containing 50, 75 and 100 ml of the medium showed slightly growth rate and enzyme yield. This suggests that lower level of aeration found to be suitable for the growth and yield of enzyme (Figure 5.1). This may be due to the fact that lower level of aeration may facilitate the suitable mycelium branching for the yield of enzyme. Further the results obtained in this experiment have shown little difference in yield of enzyme. This suggests that the aeration did not influence in the yield of enzyme. The effect of aeration on growth rate and yield of enzyme is essentially in good agreement with the findings of Bilimoria (1969).
5.3.4 Effect of NaCl on the Growth Rate to Enzyme Production

Dhevendaran and Annie (1999) suggested that minimum concentration of salts, preferably NaCl is essential for the growth and L-asparaginase production for marine Streptomyces. The effect of NaCl on the growth rate to enzyme yield was studied in different concentrations of NaCl. A level of 4 % NaCl was found to enhance the good growth and yield of enzyme (Table 5.3). This indicates that strain EPD 27 is assumed to be indigenous marine origin and the salt tolerance is inherent property not only to the strain EPD 27 but also to the enzyme.
Table 5.3 Effect of NaCl on the growth and L-asparaginase production

<table>
<thead>
<tr>
<th>Salt concentration (%)</th>
<th>Dry weight of mycelium (mg/ml)</th>
<th>L-asparaginase (IU/mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.34</td>
<td>0.21</td>
</tr>
<tr>
<td>2</td>
<td>0.57</td>
<td>0.44</td>
</tr>
<tr>
<td>3</td>
<td>1.76</td>
<td>1.84</td>
</tr>
<tr>
<td>4</td>
<td>3.84</td>
<td>1.98</td>
</tr>
<tr>
<td>5</td>
<td>2.14</td>
<td>1.63</td>
</tr>
</tbody>
</table>

5.3.5 Growth and L-asparaginase Production in Batch Fermentation

Nutritional requirements for maximal production of L-asparaginase vary between organisms. In general, the successful production of fermentation products depends on yield per volume of culture fluid. Since L-asparaginase is extracted from whole cells, efficiency in production will be determined by cell density and the amount of enzyme that each cell produces. The previous studies have shown that L-asparagine is the suitable source for production of growth and yield of L-asparaginase for Streptomyces karanatakensis (Mostafa and Salama 1979 and Mostafa 1982). In this respect, the effect of L-asparagine on growth rate to enzyme yield was studied in batch fermentation to confirm whether the strain EPD 27 L-asparaginase is nitrogen regulated as the enzyme produced by Streptomyces karnatakensis. A series of batch fermentations were carried out in 1L synthetic starch casein medium modified with different concentration of L-asparagine.

The effect of the L-asparagine on the growth rate to the yield of enzyme was tested at different point of time such as 24, 48, 72, 96 and 120 h of fermentation. The results obtained are shown in Figure 5.2 to 5.5, indicate the time course of the mycelium growth and enzyme synthesis at different concentration of L-asparagine.
Figure 5.2 Time course of mycelium growth and L-asparaginase production by strain EPD 27 in 0.4 % of L-asparaginase.

Figure 5.3 Time course of mycelium growth and L-asparaginase production by strain EPD 27 in 0.8 % of L-asparaginase.
Figure 5.4  Time course of mycelium growth and L-asparaginase production by strain EPD 27 in 1.2 % of L-asparaginase

While observing growth and yield of enzyme at 24 h, the growth and yield of enzyme was found to be similar in all the different concentrations of L-asparagines. There was a poor growth and enzyme yield. After 48 h, the
enzyme yield in 0.4, 0.8 and in 1.6% of L-asparagine was found to be lower than the growth of mycelium. On comparison, a level of 1.2% yielded a similar growth and enzyme yield. This suggests that the growth and yield of enzyme highly depend on the nitrogen source of L-asparagine.

During the course of 72 h, the 0.4% of L-asparagine did not increase the growth of mycelium and yield of enzyme. On comparison, a considerable increase in the growth of mycelium and enzyme production was observed in 0.8% of L-asparagine than in 0.4%. While at 1.2%, there was high increase in growth of mycelium and enzyme yield. It was also found that there was a linear relationship between the growth of mycelium and enzyme production. On comparison, a level of 1.6% yielded high yield of growth than that of the enzyme produced. This may be due to the fact that the L-asparagine to a concentration of 1.2% was found to enhance the mycelia growth with respect to enzyme yield.

During the course of 96 h, there was no further increase in the growth of mycelium and yield of enzyme in 0.4 and 0.8% of L-asparagine. On comparison, a remarkable increase in growth and enzyme yield in 1.2% was observed. Further, there was a decreased growth and enzyme yield of 1.6%. This suggests that a level of 1.6% L-asparagine was found to be inhibitory to both growth and yield of enzyme as compared to 1.2% of L-asparagine. While at 120 h, there was an ample increase in growth and enzyme yield in 1.2% than in other concentration. This may be due to the fact that the production of L-asparaginase was found to occur in exponential and in stationary phase. The production of L-asparaginase was found to be induced by the natural substrate L-asparagine. Hence L-asparagine is found to be suitable source for the L-asparaginase synthesis by marine Streptomyces sp strain EPD 27. Further, a level of 1.2% was found to be the optimum concentration for the growth and yield of enzyme. The growth and enzyme
yield obtained was found to be higher than Streptomyces griseus ATCC 10137 which showed 1.26 mg of dry biomass and 1.10 IU/mg of L-asparaginase (De Jong 1972) and recombinant E. coli which showed only 2.87 mg of dry biomass and 3.4 IU/mg of enzyme (Khushoo et al 2005).

The nutritional requirement of this marine strain EPD is highly relied on L-asparagine for the growth and L-asparaginase production. This suggests that L-asparaginase production is nitrogen regulated, similar to enzyme production in E. coli A-1 (Barnes et al 1977). From the Figure 5.2 to 5.5, L-asparagine is considered to be the growth limiting substrate, thus making it possible to apply the Monod equation to the analysis of batch fermentation data for L-asparaginase synthesis by strain EPD 27.

Figure 5.6  Reciprocal plots of specific growth rate versus reciprocal of limiting substrate for strain EPD 27
In this work, a maximum of 7.9 mg of dry biomass and 12.4 IU/mg of enzyme was obtained in 1.2% of L-asparagine. However, 1.6% of L-asparagine was found to be inhibitory to enzyme production and mycelium growth, it yields growth of 5.7 g/l and 2.2 IU/ml of enzyme. Hence L-asparagine was used in a Line weaver-Burk plot, ie.1/µ versus 1/S (Figure 5.6). Here µ represents the specific growth rate in the exponential growth phase, and S is the concentration of the L-asparagine which is close to the initial concentration because of the low concentration. As shown in Figure 5.6, a linear relation was observed, and the following growth rate was then obtained by using the Monod equation \( \mu = \frac{0.909 S}{1.36+S} \). Therefore, \( \mu_{\max} = 0.909 \text{ (h}^{-1} \text{)} \) saturation constant is \( K_s = 1.36 \text{ g/L} \). This indicates that the strain EPD 27 has high affinity towards the natural substrate L-asparagine for the growth and production of enzyme.

5.4 CONCLUSION

These findings suggest that the pH and temperature optima for the production of L-asparaginase were found to be the same as that for the growth of strain EPD 27. Lower level of aeration was observed to be effective for the production of L-asparaginase. NaCl to a concentration of 4% showed good growth and yield of enzyme and thereby it confirms the marine origin of the strain EPD 27. L-asparagine to a concentration of 1.2% was found to enhance high growth and yield of enzyme. L-asparagine was found to be the growth limiting substrate and \( K_s \) value of 0.516 g/L was obtained using L-asparagine. This suggests that the strain EPD 27 has higher affinity towards the substrate L-asparagine for the production of L-asparaginase. In addition, the newly discovered marine strain EPD 27 is found to be a vital source for the production of antitumour L-asparaginase in large scale.
5.5 SUMMARY AND CONCLUSIONS

The exploitation of enzymes as drugs in cancer treatment seems promising but it needs a suitable bacterial source which lacks toxin to produce sufficient quantity of enzyme pure enough to reach the goal. L-asparaginase is an effective drug in the treatment of lymphoid malignancies mainly ALL. It has been an integral part of combination chemotherapy protocols of pediatric ALL for almost three decades. Today, L-asparaginases used in the clinic are available in three preparations: two unmodified forms purified from two bacterial sources such as E. coli and Erwinia, and a PEGylated form of E. coli L-asparaginase. The main disadvantage of existing L-asparaginases has been causing the fatal effects. Hence the potential of L-asparaginase as a drug for leukemia has been a matter of discussion due to the high rate of allergic reactions exhibited by the patients who are receiving the medication of this enzyme drug. However, there is an immense demand for new bacterial source of L-asparaginase with better therapeutic properties to prevent or reduce side effects.

For an antitumour L-asparaginase to be ideally suited for use in antineoplastic therapy, it should satisfy a variety of criteria. The organism that is selected should be a non pathogenic and produce the asparaginase in high yield. It should be capable of being grown in large quantities on a simple and inexpensive medium. The procedures developed for purification of the enzyme should be rapid and simplified, providing pure enzyme in high yield. The purified enzyme should have long term stability on storage, maximal activity at a physiological pH and a $K_\text{m}$ for substrate below the concentration of substrate in the blood. Most importantly, the enzyme should produce antitumour activity on leukemic cells. In this respect, a systematic study on marine actinobacteria has been carried out for novel antitumour
L-asparaginase for therapeutic purpose. The results obtained are in good agreement with the criteria.

A total of thirty four marine actinobacteria were isolated from the sediments of Parangipettai, East coast region of India and were screened for their L-asparaginase activity. Out of thirty four marine actinobacteria isolates, 21% of isolates showed L-asparaginase activity. Among them, the strain EPD 27 was selected as a high L-asparaginase potential strain based on the amount of enzyme production.

Then it was further identified by using a combination approach which includes classical and molecular methods for the authentication. By the classical method, the cultural, morphological and biochemical characteristics of the strain EPD 27 was determined and was found to be similar to that of Streptomyces griseofuscus except in the fermentation of fructose and hydrolysis of L-asparagine. By the molecular method, 16S rRNA gene fragment of the strain EPD 27 was sequenced and it has 99.4% similarity to the other species of genus Streptomyces. The sequence was submitted to GENBANK, accession number FJ 951436 and thus the strain EPD 27 was ultimately identified as marine Streptomyces sp strain EPD 27.

The crude enzyme prepared from the strain EPD 27 was used for purification of L-asparaginase. In this present study a modified purification procedure was developed from the method Davidson et al (1977). This modified method essentially includes a series of column chromatography such as DEAE cellulose, CM sepharose and sephadex G 200. DEAE column was found to be important for separation of L-asparaginase and L-glutaminase and very effective in initial step of purification. Followed by enrichment and polishing of L-asparaginase in CM sepharose affinity column and in gel filtration column respectively. The sephadex gel purified high active fraction showed a specific activity of 416.3 IU/mg protein with 48.4% of recovery.
The purified enzyme was lyophilized for further characterization of the enzyme. The homogeneity of enzyme was confirmed in HPLC and was found to be suitable for the evaluation of antitumour activity.

The purified enzyme was characterized with respect to therapeutic purpose. The optimum pH and temperature of enzyme activity was found to be pH 8.6 and 60 °C respectively. Electrophoretic analysis of the enzyme revealed that it has apparent molecular weight of 130 kDa. The enzyme possessed higher affinity towards its natural substrate L-asparagine as it gives an apparent $K_m$ of $2.78 \times 10^{-3}$ M. Further the antitumour activity of the enzyme was analyzed in four different liquid and solid cancer cell lines such as JURKAT (acute T cell leukemia), HL 60 (acute myeloid leukemia), K562 (chronic myeloid leukemia) and HT 29 (colon adenocarcinoma). It was found that the enzyme possesses cytotoxic effects towards all cell lines. The percentage viability inhibition was about 150% on JURKAT, 130% on HL 60 and 120% on K562 and a considerable about 80% on HT 29. In this present investigation, marine Streptomyces sp strain EPD 27 and its interesting novel antitumour L-asparaginase is discovered. This prompted to use the strain for large scale production of antitumour L-asparaginase. Further, the fermentation kinetics of the enzyme production was carried out in batch fermentation.

The carbon and nitrogen sources for the production of enzyme by Streptomyces have been reported in detail. Yet the fermentation kinetics of the L-asparaginase production has not been reported. A series of experiments in Erlenmeyer flask and in fermenter were carried out using starch casein medium modified with L-asparagine. Synthetic starch casein medium with 1.2% of L-asparagine was found to be suitable for the production of L-asparaginase by marine Streptomyces sp strain EPD 27. The optimum pH and temperature for growth and yield of enzyme was found to be similar.
Gentle aeration was found to be sufficient for the growth and enzyme production.

These findings suggest that marine sediments of Parangipettai, East coast region of India are found to be suitable for the isolation of novel marine actinobacteria. Marine Streptomyces are found to be wide and persistent populations of new marine actinobacteria in sediments. The indigenous marine Streptomyces of Parangipettai are found to be an potential source for screening of L-asparaginase producers. A combined screening procedure such as qualitative and quantitative assays was found to be effective in rapid identification of the L-asparaginase producers. In this present investigation, a high L-asparaginase potential marine Streptomyces sp strain EPD 27 was discovered from sediments of Parangipettai. Further the antitumour L-asparaginase purified from marine Streptomyces sp strain EPD 27 meets all the important criteria to be an alternative antitumour agent. Hence it is concluded that marine Streptomyces sp strain EPD 27 discovered is a novel source for potential antitumour L-asparaginase.

5.6 SCOPE FOR FUTURE RESEARCH

The animal model studies such as immunosuppressive and toxic effects of the enzyme and enzyme half life would help determine the use of enzyme in human beings. Further the pharmacological studies such as measurement of serum asparagine in the presence of the enzyme, and cytotoxic activity of the enzyme in the presence of various inhibitors would help to authenticate the use of this marine L-asparaginase as an anticancer drug.