L-ASPARAGINASE ACTIVITY IN MORAXELLA SP.

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ABSTRACT

L-asparaginase activity and growth in Moraxella sp. W2 were studied under laboratory conditions. The bacterium showed one pH optimum at 5.6. The influence of aminoacids, carbon sources and sodium chloride showed wider variations. Heavy metals could also exert inhibition over the growth and enzyme activity.

Key-words: Asparaginase, enzyme, Moraxella

L-asparaginase (L-asparagine amino hydrolase 9.5.1.1.) is an enzyme applied for the treatment of tumour and of acute lymphatic leukaemia of human beings. Occurrence of this enzyme has been reported in bacteria, fungi and actinomycetes (Balakrishnan Nair, Selvakumar, Chandramohan and Natarajan, 1977; Selvakumar, 1979). However, it was found that not all the L-asparaginase from different sources did exhibit both antitumour and antileukaemic properties. Information on the occurrence of L-asparaginase enzyme in microorganisms on the west coast of India is lacking. Hence the present study is an attempt to denote the L-asparaginase activity in certain bacteria of the retting ground of Kerala coastal water.

Water, sediment and three months old retting sources were collected from Anchuthengu, Edava and Murukkampuzha regions. All the samples were brought to the laboratory in sterile containers taking all precautions to prevent any further external contaminations, standard pour plate technique was followed using nutrient asparagine medium (peptone - 0.1 g; sodium chloride - 0.5 g; potassium dihydrogen phosphate - 0.2 g; L-asparagine - 0.2 g; phenol red - 0.2%; Agar - 2 g; 50% seawater 100 ml; pH - 7.0). The petri-dishes were incubated at room temperature (28 ± 2°C) for five days. The appearance of pink colour around the colonies were noted as L-asparaginase positive cultures. All these cultures were screened by the conventional method to confirm the L-asparaginase activity (Selvakumar, 1979).

The packed cells were suspended in distilled water to a population of $1 \times 10^8$ C.F.U. (Colony Forming Unit). From this stock culture, 0.2 ml of the young cultures were independently inoculated into 5.5 ml of the nutrient broth. The medium was incorporated with 0.2 ml of 1% L-asparagine. They were incubated at room temperature for 48 hr. After the incubation period was over the bacterial biomass was estimated by measuring the turbidity in Spectronic-20 at a wave length of 600 nm.
L-asparaginase activity was measured by adding 0.5 ml of Nessler's reagent into 5.5 ml culture broth after growing for 48 hr. Within a few minutes yellow colour was developed. The whole sample was centrifuged and supernatant was read in Spectronic-20 at a wave length of 490 nm (Selvakumar, Chandramohan and Natarajan, 1977) and suitable control was maintained throughout the period of investigation. The standard was prepared with ammonium chloride. The activity was expressed as μg ammonia/ml/hr.

To study the enzyme activity in the bacterial culture the medium was prepared in the buffered solution ranging from pH 4 to 11. Appropriate temperature was maintained with refrigerator and incubator.

To study the tolerance of bacterial strain towards sodium chloride, the medium prepared with distilled water was incorporated with sodium chloride at varying concentrations ranging from 0.1 to 5%. Simultaneously control was maintained without sodium chloride.

In order to understand the influence of organic compounds, amino acids such as methionine, threonine, cysteine, L-glutamic acid and tryptophan were selected and prepared at a concentration of 0.8 mg/ml. Carbon sources such as glucose, lactose,

<table>
<thead>
<tr>
<th>Sl. No.</th>
<th>Bacteria</th>
<th>Stock Culture Number</th>
<th>Source</th>
<th>L-asparaginase activity (μg/ml/hr)</th>
<th>Growth (OD at 600 nm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td><em>Vibrio</em> sp.</td>
<td>S1</td>
<td>Sediment</td>
<td>50.0</td>
<td>0.17</td>
</tr>
<tr>
<td>2.</td>
<td><em>Bacillus</em> sp.</td>
<td>S1</td>
<td>Sediment</td>
<td>56.5</td>
<td>0.62</td>
</tr>
<tr>
<td>3.</td>
<td><em>Micrococcus</em> sp.</td>
<td>H2</td>
<td>Husk</td>
<td>11.5</td>
<td>0.35</td>
</tr>
<tr>
<td>4.</td>
<td><em>Micrococcus</em> sp.</td>
<td>W1**</td>
<td>Water</td>
<td>56.5</td>
<td>0.52</td>
</tr>
<tr>
<td>5.</td>
<td><em>Bacillus</em> sp.</td>
<td>S2</td>
<td>Sediment</td>
<td>15.5</td>
<td>0.75</td>
</tr>
<tr>
<td>6.</td>
<td><em>Moraxella</em> sp.</td>
<td>H1***</td>
<td>Husk</td>
<td>45.0</td>
<td>0.325</td>
</tr>
<tr>
<td>7.</td>
<td><em>Micrococcus</em> sp.</td>
<td>S1</td>
<td>Sediment</td>
<td>18.2</td>
<td>0.45</td>
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<tr>
<td>8.</td>
<td><em>Micrococcus</em> sp.</td>
<td>H2</td>
<td>Husk</td>
<td>68.5</td>
<td>0.10</td>
</tr>
<tr>
<td>9.</td>
<td><em>Bacillus</em> sp.</td>
<td>H2</td>
<td>Husk</td>
<td>68.5</td>
<td>0.58</td>
</tr>
<tr>
<td>10.</td>
<td><em>Bacillus</em> sp.</td>
<td>H3</td>
<td>Husk</td>
<td>77.5</td>
<td>0.54</td>
</tr>
<tr>
<td>11.</td>
<td><em>Moraxella</em> sp.</td>
<td>H3</td>
<td>Husk</td>
<td>155.0</td>
<td>0.62</td>
</tr>
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<td>12.</td>
<td><em>Corynebacterium</em> sp.</td>
<td>S2</td>
<td>Sediment</td>
<td>61.0</td>
<td>0.10</td>
</tr>
<tr>
<td>13.</td>
<td><em>Arthrobacter</em> sp.</td>
<td>S1</td>
<td>Sediment</td>
<td>60.0</td>
<td>0.70</td>
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<td>14.</td>
<td><em>Moraxella</em> sp.</td>
<td>H2</td>
<td>Husk</td>
<td>27.5</td>
<td>0.70</td>
</tr>
<tr>
<td>15.</td>
<td><em>Micrococcus</em> sp.</td>
<td>W2</td>
<td>Water</td>
<td>58.5</td>
<td>0.10</td>
</tr>
<tr>
<td>16.</td>
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<td>S2</td>
<td>Sediment</td>
<td>68.5</td>
<td>0.90</td>
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<tr>
<td>17.</td>
<td><em>Bacillus</em> sp.</td>
<td>W2</td>
<td>Water</td>
<td>12.5</td>
<td>0.85</td>
</tr>
<tr>
<td>18.</td>
<td><em>Micrococcus</em> sp.</td>
<td>S1</td>
<td>Sediment</td>
<td>18.5</td>
<td>7.5</td>
</tr>
<tr>
<td>19.</td>
<td><em>Moraxella</em> sp.</td>
<td>W2</td>
<td>Water</td>
<td>155.0</td>
<td>1.25</td>
</tr>
</tbody>
</table>

*S - Sediment sample, **W - Water sample, ***H - Husk sample
sucrose and starch were also prepared at a concentration of 1%. Toxicity of heavy metals such as mercury, copper, zinc and arsenic was studied by incorporating them in the medium independently at 2 ppm concentration.

Asparaginases could be obtained from a few marine bacterial strains such as *Aeromonas hydrophylia*, A. *liquifaciens* and *Vibrio* sp. (Selvakumar, 1979). Randomly selected nineteen isolates from different sources were subjected to L-asparaginase activity and their growth. They could exhibit activity and growth (Table I). Invariably all the strains synthesised this enzyme during growth.

Of all these *Moraxella* sp. W2 was selected for the experimental studies because it exhibited elevated activity of this enzyme and profused growth. The cultural conditions studied were pH, temperature, sodium chloride, carbon sources, aminoacids, inorganic phosphate and sulphate, and effect of heavy metals on the enzyme activity as well as on growth.

![Graph showing pH activity and growth](image)

Fig. 1. L-asparaginase in *Moraxella*

The results showed one pH optimum at 5.6 (Fig. 1). However, the growth was maximum at pH 8.0. It is interesting to note that minimum enzymatic activity was recorded at this pH (pH 8.0). This clearly indicates that the growth of the bacterium and its enzyme activity were independent. Selvakumar, Chandramohan and Natarajan (1977) and Selvakumar (1979) observed two pH optima in mangrove sediments at pH 6.2 and 8.7, and a single optimum pH in *Vibrio* sp. at 8.7. Possibly the extracellular secretion of microbial cell or intracellular enzymes released after the death of the cell may be the reason for the peak at pH 5.6. The increased trend of activity towards alkaline range showed close similarities with *Streptomyces griseus* (Dejong, 1972).

It was noticed that maximum activity and optimum growth were at 37°C (Fig. 2). However, Selvakumar, Chandramohan and Natarajan (1977) and Selvakumar (1979) reported the maximum activity at 80°C in marine sediment and at 60°C in *Vibrio* sp. It is interesting to note that in the natural environment, the activity and the growth
were at an elevated level since the temperature of the retting ground region ranged from 30 to 31°C. Under laboratory condition, the retting process was accelerated with certain bacterial cultures. It showed exothermic reactions and increase in temperature. Differential type of enzyme producing micro-organisms were recorded in the retting ground (Dhevendran, Maya and Natarajan, 1992).

The minimum concentration of 0.1% sodium chloride enhanced the enzyme activity whereas the total cell mass production was maximum at 0.5% sodium chloride (Fig. 3). Above this level the synthesis of the enzyme and the growth of bacterium retarded. In the natural environment the salinity ranged between 5 and

![Graphs showing temperature and sodium chloride concentration effects on activity and growth.]

Fig. 2 & 3. L-asparaginase in *Moraxella*

**Table II. Effect of amino acids on the L-asparaginase activity of *Moraxella***

<table>
<thead>
<tr>
<th>Amino acids</th>
<th>Activity (μg/ml/hr)</th>
<th>Growth (OD at 600 nm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Methionine</td>
<td>1.0</td>
<td>0.15</td>
</tr>
<tr>
<td>Theonine</td>
<td>4.0</td>
<td>0.12</td>
</tr>
<tr>
<td>Cystein</td>
<td>5.6</td>
<td>0.28</td>
</tr>
<tr>
<td>L-glutamic acid</td>
<td>2.1</td>
<td>0.25</td>
</tr>
<tr>
<td>Tryptophan</td>
<td>1.1</td>
<td>0.23</td>
</tr>
</tbody>
</table>

**Table III. Effect of carbon source on L-asparaginase activity.**

<table>
<thead>
<tr>
<th>Carbon Sources</th>
<th>Activity (μg/ml/hr)</th>
<th>Growth (OD at 600 nm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose</td>
<td>2.4</td>
<td>0.95</td>
</tr>
<tr>
<td>Lactose</td>
<td>1.0</td>
<td>0.69</td>
</tr>
<tr>
<td>Sucrose</td>
<td>4.3</td>
<td>1.00</td>
</tr>
<tr>
<td>Starch</td>
<td>2.9</td>
<td>0.33</td>
</tr>
</tbody>
</table>
15%. It could be presumed that the L-asparaginase might be synthesised at lower concentration of sodium chloride. The reduced activity at higher concentration may be due to the formation of certain inhibitors with sodium chloride during growth. It may be mentioned here that phosphate and nitrate at higher concentration inhibited soil asparaginase (Mouraret, 1965). Selvakumar, Chandramohan and Natarajan (1977) noticed similar pattern of enzyme activity in marine sediments. For a marine or estuarine bacterium, the minimum concentration of salts preferably sodium chloride is essential for its growth and activity.

The results showed that cysteine comparatively enhanced the L-asparaginase activity and growth (Table II). Denneen and Carver (1968) observed that the synthesis of antibiotic, cephalosporin C from Cephalosporium acremonium was stimulated by cysteine in the growing basal medium.

Table III showed that sucrose enhanced the L-asparaginase activity and growth in Moraxella sp. W2. Selvakumar (1979) tested L-asparaginase activity with carbon sources and found that lactose had inhibiting effect in marine Vibrio sp. Similarly in our observation the enzyme activity was minimum with lactose.

Table IV. Effect of heavy metals on L-asparaginase activity.

<table>
<thead>
<tr>
<th>Heavy metals (2 ppm)</th>
<th>Activity (µg/ml/hr)</th>
<th>Growth (OD at 600 nm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hg</td>
<td>5.0</td>
<td>0.13</td>
</tr>
<tr>
<td>Cu</td>
<td>2.0</td>
<td>0.19</td>
</tr>
<tr>
<td>Zn</td>
<td>13.0</td>
<td>0.22</td>
</tr>
<tr>
<td>As</td>
<td>13.2</td>
<td>0.23</td>
</tr>
</tbody>
</table>

The effect of various divalent metal ions on enzyme activity was also studied (Table IV). The metal ions were in contact with the enzyme during the incubation period. The enzyme did not exhibit any requirement but was inhibited by added metals. These have strong inhibiting effect on L-asparaginase of marine Vibrio sp. associated with shellfish Anadara rhombica (Selvakumar, 1979).

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REFERENCES


