Glutaminases are important enzymes playing a major role in biogeochemical cycling of carbon and nitrogen in natural waters and sediments in the ecosystem (Salis and Burns, 1989). Glutaminase (L-Glutaminase amidohydrolase EC 3.5.1.2) contributes significantly to the nitrification process by releasing ammonia from L-Glutamine accumulated in the natural environments due to the death and decay of a variety of plants and animals. Besides being important in the biogeochemical cycling of organic material, glutaminases have shown appreciable activity in the clinical trials for antitumor or antileukaemic treatment alone or in combination with other amidases like asparaginases (Roberts et al., 1972; Salis et al., 1974; Benny and Kurup 1991). Hardly there is any report of thermostable glutaminase. However, few glutaminase dehydrogenases have been studied from the archaebacterial kingdom. Glutamate dehydrogenases from *Sulfolobus sulfuratus* and *Pyrococcus furiosus* are the only glutamate dehydrogenases from extremely thermophilic archaebacteria that have been described (Hudson et al., 1973).

Thirty-five bacteria from three hot springs of Orissa with a temperature ranging between 44 and 60°C have been isolated (Rath and Subramanyam, 1994). The three hot springs investigated are open environments with potential sources of protein and nitrogenous compounds of animal and plant origins. Therefore, the organisms isolated from these environments could be potential producers of glutaminases or other amide hydrolases in the ecosystem. With this advent, the isolates were studied for glutaminase activity.

The isolates were screened for glutaminase activity in liquid medium. Glutaminase medium contained peptone - 1 g, sodium chloride- 5 g, monopotassium dihydrogen phosphate - 2 g, phenol red (aqueous solution) - 6 ml and L-glutamine (Loba Chemie, Bombay) - 20 g/l, and the pH was adjusted to 7. Medium (2 ml each) was dispensed into test tubes and autoclaved at 121°C for 10 minutes. Twenty microlitres of freshly-grown cultures of the test bacteria were inoculated.
into the medium and the tubes were incubated at 37, 45 and 60°C for 7 days. A change in colour from amber yellow to pink or red was an indication of positive glutaminase activity.

From the preliminary studies, it was observed that six strains (SRT-6, SRT-7, SRT-10, SRT-16, SRT-17 and SRT-18) showed a relatively early (2 d) glutaminase activity. The change in the colour of the medium was due to the change in pH because of the release of ammonia from L-glutamine which was degraded in the presence of glutaminase. Strains SRT-6, SRT-7 and SRT-10, and SRT-16, SRT-17, SRT-18 were identified as *Pseudomonas* sp. and *Bacillus* sp., respectively. The three strains SRT-16, SRT-17 and SRT-18 which showed glutaminase activity at high temperatures were studied further. The bacteria were grown in Nutrient Broth (Hi-Media Laboratories, Bombay) for 24 hours and concentrated by centrifugation (10,000 x g), washed with sterile distilled water and the suspension adjusted to an optical density of 0.4 at 600 nm. This cell suspension was used as the enzyme preparation. Glutaminase activity was assayed following Ramaiah and Chandramohan (1992) substituting L-Glutamine for L-Aspargin in the assay mixture.

While the enzyme activity was measured in terms of release of ammonia by incubating the assay mixture at high temperature (40, 50 and 60°C), it was found that all the three strains showed a very good enzyme activity at 40 and 50°C (Table 1). The activity was markedly lower at 60°C, indicating the optimal activity of the enzyme to be around 50°C. Activity at higher temperature is an indication of the thermostable nature of the enzyme.

**Table 1. Glutaminase activity (µmoles NH₃/h) of host-spring bacteria at different temperatures (°C)**

<table>
<thead>
<tr>
<th>Strains</th>
<th>Activity at temperature</th>
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<tbody>
<tr>
<td></td>
<td>40</td>
</tr>
<tr>
<td>SRT-16</td>
<td>1.3</td>
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<tr>
<td>SRT-17</td>
<td>1.7</td>
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<tr>
<td>SRT-18</td>
<td>1.2</td>
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</table>

The effect of incubation time on the activity of glutaminase was studied by assaying enzyme at 6-h intervals up to 48 hours. The results showed that the enzyme production increased gradually over time with SRT-16 and SRT-17, whereas in the case of SRT-18, a linearity in the activity was observed up to 36 hours (Fig. 1). With all the 3 strains, as much as 97% of the total activity was recorded within 24-36 hours. After this period, no substantial increase in the activity was noticed.
Fig. 1. Effect of incubation time on glutaminase activity

Fig. 2. Effect of pH on glutaminase activity

The effect of pH on the activity of the enzyme was also studied employing different buffers (0.1 M acetate buffer; 0.1 M citrate buffer; 0.1 M tris HCl buffer), of different pH. Strains SRT-18 and SRT-17 showed two pH optima, at pH 6 and 9, whereas SRT-18 showed peak activity at pH 6 though its activity was discernible even up to pH 9 (Fig. 2). A complete loss of enzyme activity with all the three strains was observed at pH 10. The two pH optima of SRT-16 and SRT-17 possibly imply the presence of two different enzymes acting simultaneously. Similar results have been reported earlier (Dharmaraj et al., 1977). Among the buffers used, acetate buffer seems more suitable than the other two. Glutaminase of a Gram-positive coccus, Sarcina sp. isolated from soil was found to have an optimum activity around pH 8.4 (Holcenberg and Dolwy, 1973). Hughes and Williamson (1952) reported glutaminases having optimal activities at about pH 5 from Escherichia coli, Proteus morganii and Clostridium welchii.

When the enzyme activity was studied at different concentrations (1-10%) of sodium chloride, it was observed that enzyme activity was not affected up to 10% of sodium chloride in the assay mixture. Interestingly, Kiss (1961) could observe the glutaminase activity of Gram-negative bacteria isolated from soil even in the presence of 25% sodium chloride in the assay mixture. An attempt was made to observe the effect of substrate concentration on the enzyme activity. It was observed that 180 µ moles of substrate (L-Glutamine) in the assay mixture was optimum for the enzyme activity.
After screening a large number of bacterial strains from different sources, it was concluded that effective production of glutaminase or other amidases depends on the habitat (Hvozyak et al., 1973; Dharmaraj et al., 1977). It has also been shown that the presence of this enzyme in the natural isolates is random, regardless of the source of their origin (Selvakumar, 1979). Our results indicate thermostable glutaminase activity in some of the bacteria isolated from the hot springs. Besides its importance as an antitumor agent, glutaminase contributes significantly providing a link between carbon and nitrogen metabolism in the ecosystems. The hot springs of Orissa have so far not been explored in terms of their microbial wealth. Explorative studies such as these are a prerequisite for tapping the biotechnological potential of natural resources.

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REFERENCES