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Short Communication

Microbial L–asparaginase from mangroves of Andaman Islands

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Bacterial strains from mangroves of Andamans were assayed for L-asparaginase enzyme. One hundred and eight (54%), of total 200, isolates synthesized L–asparaginase, growing at *p*H 7.2 and 37°C temperature. Only two gram negative strains appeared to be potent for large-scale production.

Andaman and Nicobar group of islands occupy 25% of the total mangrove area, in India. They play important role in nutrient recycling. L-asparaginase is extensively used in the treatment of tumors and leukemia[1]. Several marine microorganisms especially estuarine bacteria are potential source of L-asparaginase enzyme and the halophilic (bacterial growth is accelerated or dependent on the high salt content) nature of the bacteria can be exploited for large-scale industrial production[2]. There are reports of studies on microbial L-asparaginase from India[2-4], however, no information is available from Andaman and Nicobar Islands. Hence, bacteria isolated from the litterfall and sediment samples were screened for the presence of L-asparaginase.

Twenty each of litterfall and soil samples were collected from mangroves :(*Rhizophora*, *Avicenia* and *Nypa*) inhabited areas during 1996-99. Samples, brought in sterile tubes, were cultured first in Zobell marine broth and later in Zobell marine agar 2116 E (Hi media, Bombay). The mixed bacterial colonies were purified by repeated streaking in the same medium and purified bacterial isolates were preserved at -86^{o}C in Zobell broth containing 10% glycerol. The presence of L-asparaginase enzyme in the bacterial strains was initially tested by using marine agar medium incorporated with 0.2% L-asparagine and a few drops of phenol red indicator. The colonies around which the medium changed from yellow to red color due to the production of ammonia were selected for quantitative enzyme assay. All the selected isolates were cultured in the media, *p*H of which was adjusted to 4, 7.2, 10 and 12 in quadruplicate. One set each of all the cultures in medium at each *p*H were incubated at various temperatures viz. 15°, 24°, 37° and 45°C to determine the optimum *p*H and temperatures for growth.

Bacterial cells grown in 10 ml Zobell broth for 24 hr was concentrated by centrifugation at 10000 rpm for 10 minutes at 4°C and washed twice with filter sterilized seawater. The cells were suspended in 1 ml of sterile seawater and subjected to 3 cycles of slow freezing and quick thawing and used as crude enzyme preparations (CEP) for enzyme assay[4]. To the 0.25 ml of crude enzyme prepared, 1.25 ml of 0.2 *M* borate buffer (*p*H 8) and 0.5 ml of 0.04 *M* L – asparagine in borate buffer were added and incubated at 35°C for 30 min. The reaction was stopped by the addition of 0.5 ml of 15% trichloroacetic acid (TCA). The mixture was again centrifuged at 4000 rpm for 20 min and one ml of separated supernatant was mixed with 4 ml distilled water free of ammonia. Nesslers reagent was latter added and the colour intensity was read at 425 nm. The ammonia content was estimated using standard ammonium chloride solution and protein content of the enzyme preparation was estimated by Lowry's method[5]. One IU of the enzyme activity (IU mg^{-1} protein) is equivalent to 1 μ mole NH_3 liberated per minute.

Out of 200 bacterial isolates purified and tested, only 108 (54%) were L–asparaginase producers. Notably, out of the L–asparaginase producers, a very high percentage of bacteria, 87.9% and 33.3% were

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Table1— L-asparaginase producing bacteria isolated from mangroves of Andamans

Specific activity (IU mg^{-1} protein)	Staining pattern			Total
	Gram (+) ve	Gram (-) ve	Gram variables	
0.0-0.1	9	15	2	26 (24%)
0.1-0.2	24	30	2	56 (51.8%)
0.3-0.4	7	14	3	24 (22.2%)
0.4-0.5	-	2	-	2 (1.8%)
Total	40 (37.0%)	61 (56.5%)	7 (6.5%)	108

capable of growing at alkaline *p*H of 10 and 12 respectively and 6.6% were also found to be thermotolerant and hence would be preferred in industry. After repeated staining using fresh and old cultures, the gram's reaction was difficult to spell out in gram variables. Earlier, Shome *et al*.[6] reported 76.3% bacteria as gram positive out of 38 bacterial isolates obtained from mangrove areas of South Andaman.

In quantitative L–asparaginase assay, 24% of the isolates showed minimum activity below 0.1 IU mg^{-1} protein , 51.8% showed an activity between 0.1 - 0.2 IU mg^{-1} protein and 22.2% of the isolates showed more than 0.3 IU mg^{-1} protein (Table 1). Only two gram negative isolates growing at *p*H 7.2 and 37°C temperature showed the highest activity, between 0.4 to 0.5 IU mg^{-1} protein. Benny & Muraleedharan[3] reported more activity of L-asparaginase in *Aeromonas* followed by *Pseudomonas*, *Vibrio* and *Alkaligens*, isolated from molluscun species compared to those from estuarine soil sediments. Ramaiah & Chandramohan[4] reported higher secretion of L-asparaginase by 43 strains of luminous bacteria, belonging to 4 species isolated from marine samples than from other non-luminous bacteria.

In the present study, the gram negative bacteria showed higher L-asparaginase activity than gram positive bacteria isolated from mangrove soils. However, strains isolated from seawater particularly from tidal creeks appear to be potential sources of L-asparaginase[7]. The random screening in the present study showed that, there is good proportion of L-asparaginase harbouring bacteria in the mangrove dominated areas of Andamans. Besides being important in the biogeocycling of organic material, bacterial asparaginase have shown appreciable activity in the clinical trials for tumor/leukaemic treatments[8]. However the anti-tumor potential of L-asparaginase produced by these marine microbes in the present study is yet to be ascertained.

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