Specimens examined:

1. *Chaetopatella longiciliata* Hino and Katumotto, on *Sasa kurilensis* Makino et Shibata, Mt. Nyutozon, Prov. Ugo. by H. Muroi on August 4, 1957. ex Herb. IMI 110665 (Holotype).
2. On culms of *Bambusa* sp Munnar, Kerala, India, collected by J. Muthumary, 2–3–1978, Herb. MUBL. 2920.

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1. Sutton, B. C., *The Coelomycetes*, Commonwealth Mycological Institute, Kew, England, 1980.

## ASPARAGINASE PRODUCTION BY SOME BACTERIA

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ASPARAGINASE an antileukemia agent in mice and rats[1–3], has been exploited in the treatment of neoplastic cell in man[4]. However, only a certain type of asparaginase is active against lymphomonas[5–7]. Hence, it was considered worthwhile to screen some of the common bacteria for production of asparaginase to find a good source of this enzyme.

The bacteria were grown in 25 ml nutrient agar medium (Bacto beef extract 3 g; peptone 5 g and distilled water 1 l) contained in 100 ml Erlenmeyer conical flasks at 37°C. At the end of every 12 hr incubation period, a set of flasks were harvested and the L-asparaginase was assayed[8].

To 0.1 ml of enzyme solution, 0.9 ml of 0.1 M sodium borate buffer (pH 8.5) and 1 ml of 0.04 M L-asparagine solution were added and incubated for 20 min at 37°C. The reaction was stopped by the addition of 0.5 ml of 15% trichloro acetic acid. After centrifugation, 0.1 ml portion of the supernatant fluid was diluted to 8 ml with distilled water and treated with 1.0 ml of Nessler's reagent and 1.0 ml of 2.0 M NaOH and incubated for 15 min. The intensity of colour thus developed was read at 500 nm. The amount of ammonium liberated was read from a standard curve prepared from a solution of ammonium sulphate. One international unit (IU) of L-asparaginase is that amount of enzyme which liberates 1 $\mu$mol of ammonia$^{-1}$ at 37°C.

Table 1 reveals that all the bacteria under investigation secreted asparaginase which, however, varied significantly in the degree of production with the bacterium. *S. albus* was found to be an efficient producer of asparaginase which showed increasing trend till the 60th hr of incubation. *Klebsiella* sp was poor in secretion of asparaginase and it started secreting the enzyme only after 24 hr of incubation and the activity was constant up to 108 hr of incubation. *Bacillus subtilis* followed by B. polymyxa

**Table 1** *Asparaginase[a] activity and growth[b] of different bacteria in nutrient broth*

| Name of the bacterium | | Incubation period (hr) | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | 12 | 24 | 36 | 48 | 60 | 72 | 84 | 96 | 108 |
| *E. coli* | Growth | 0.07 | 0.19 | 0.32 | 0.34 | 0.37 | 0.40 | 0.32 | 0.32 | 0.32 |
| | L-asparaginase | — | 14.4 | 57.6 | 72 | 86.6 | 86.6 | 86.6 | 43.2 | 43.2 |
| *K.* sp | Growth | 0.05 | 0.11 | 0.19 | 0.21 | 0.23 | 0.35 | 0.35 | 0.32 | 0.30 |
| | L-asparaginase | — | — | 14.4 | 34.7 | 57.6 | 43.2 | 43.2 | 43.2 | 43.2 |
| *P. vulgaris* | Growth | 0.03 | 0.07 | 0.14 | 0.17 | 0.19 | 0.29 | 0.32 | 0.28 | 0.26 |
| | L-asparaginase | — | — | 21.6 | 21.6 | 64.8 | 64.8 | 72.0 | 86.6 | 86.6 |
| *B. polymyxa* | Growth | 0.06 | 0.11 | 0.16 | 0.28 | 0.33 | 0.43 | 0.36 | 0.32 | 0.32 |
| | L-asparaginase | — | — | 28.8 | 72.0 | 129.6 | 129.6 | 129.6 | 129.6 | 144.0 |
| *B. subtilis* | Growth | 0.04 | 0.08 | 0.13 | 0.13 | 0.14 | 0.31 | 0.33 | 0.35 | 0.34 |
| | L-asparaginase | — | — | — | — | 43.2 | 43.2 | 144.0 | 144.0 | 187.2 |
| *S. albus* | Growth | 0.07 | 0.15 | 0.26 | 0.38 | 0.43 | 0.61 | 0.63 | 0.52 | 0.49 |
| | L-asparaginase | 7.2 | 14.4 | 57.6 | 202.0 | 316.8 | 273.6 | 259.2 | 244.8 | 244.8 |

[a] In international units; [b] Growth expressed in O.D. at 650 nm.

were also efficient in the production of asparaginase. *B. subtilis* secreted this enzyme only after 48 hr of incubation. *E. coli* which is the only source of this enzyme, was found to be much inferior to *S. albus*. There was no correlation between vegetative growth and asparaginase activity of different bacteria.

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1. Broome, J. D., *J. Exp. Med.*, 1963, **118**, 99.
2. Kidd, J. G., *J. Exp. Med.*, 1953, **98**, 565.
3. Mashburn, L. T. and Wriston, J. C., *Arch. Biochem. Biophys.*, 1964, **105**, 450.
4. Oettgen, H. F., *et al, Cancer Res.*, 1967, **27**, 2619.
5. Campbell, H. A., Old, L. J. and Boyse, E. A., *Proc. Am. Assoc. Cancer Res.*, 1964, **5**, 1.
6. Roberts, J., Prager, M. D. and Bachynsky, N., *Cancer Res.*, 1966, **26**, 2213.
7. Schwartz, J. H., Reeves, J. Y. and Broome, J. D., *Proc. Natl. Acad. Sci. USA*, 1966, **56**, 1516.
8. Peterson, R. E. and Ciegler, A., *Appl. Microbiol.*, 1969, **18**, 64.

## BRANCHING DEVELOPMENT OF THE BLUE-GREEN ALGA *MASTIGOCLADUS LAMINOSUS* COHN AT DIFFERENT pH LEVEL

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A branched blue-green alga *Mastigocladus laminosus* Cohn, found growing in the main tank and over flows of the hot spring Taptapani (Orissa, India) displayed variable branching frequencies. The yearly mean temperature and pH of the spring water was 44°C and 9.0 respectively. Hydrogen-ion-concentration, an important factor of hot spring water, appears to control the degree of branching of this organism. Experiments were designed to find out the relation between growth and branching development of the *Mastigocladus* strain at different pH levels of the culture in the laboratory.

The blue-green alga *M. laminosus* was isolated and the unialgal culture of the organism was obtained by plating method[1]. Axenic cultures were obtained following the method described earlier[2]. The alga was grown at 30±1°C (by repeated cultivation the alga gradually adapted to grow at this lower temperature in comparison to its natural habitat) under continuous light from daylight fluorescent tubes at an intensity of 2400 lux in Allen and Arnon's medium[3] with trace elements as used by Fogg[4]. Growth experiments at different pH levels of the culture were performed in 100 ml Erlenmeyer flasks containing 25 ml of nitrogen-free medium. The required pH of the culture media was obtained and adjusted from time to time by asceptic addition of a few drops of 0.1 N NaOH or 0.1 N HCl. An equal volume of exponentially growing alga (equivalent to 1 mg dry weight) was inoculated to the experimental flasks and the cultures were harvested after 15 days of incubation. Growth was estimated on dry weight basis[5]. Frequency of branching was calculated by counting the number of cells at intervals between branch initials; mean values of 30 readings ±S.D. was plotted.

Results on the growth and branching development of *M. laminosus* at different pH levels of the cultures (5, 6, 7, 7.5, 8, 8.5, 9, 9.5, 10, 11) are given in the figure. No growth was observed at the pH level of 5. Brock[6] also reported that blue-green algae were absent from a wide variety of acidic environments and in enriched cultures at less than pH 5. The rate of growth of *Mastigocladus* was not substantial at pH 6. Further increase in pH of the media encouraged the growth of the alga and a maximum was obtained at

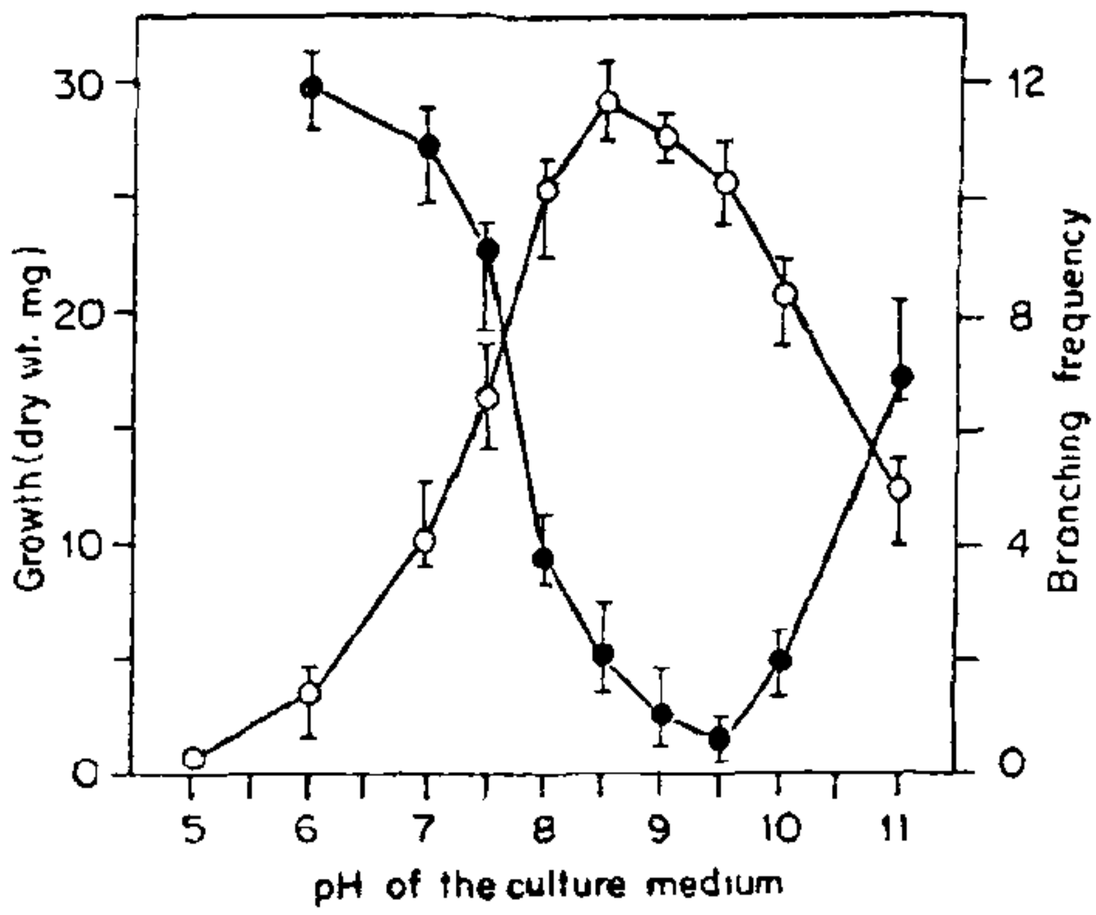


**Figure 1.** Growth and branching frequency of *M. laminosus* Cohn at different pH levels of the culture medium. Growth (○——○); Branching frequency (●——●).