

## Optimization of Culture Conditions for Protease Production by *Bacillus licheniformis* M27

Kirubaharan, T., Senthuran, A., and Vasanthi, A.

This study was aimed to improve the protease production by *Bacillus licheniformis* M27 (CFTRI, Mysore). The bacterial cells grown in fermentation medium at 42° C (100rpm) produced maximum protease activity ( $5.64 \times 10^5 \text{Uml}^{-1}$ ) at 84h. The fermentation medium contained ( $\text{gl}^{-1}$ )  $(\text{NH}_4)_2\text{SO}_4$ , 2.5; peptone, 2.0; glucose, 9.0;  $\text{Na}_2\text{HPO}_4$ , 8.0;  $\text{KH}_2\text{PO}_4$ , 4.0;  $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ , 0.5; and  $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$ , 0.025. Culture conditions were optimized to improve the protease production. The Shaking speed of culture was varied from 75 to 200rpm and the optimum shaking speed was 100 rpm ( $5.64 \times 10^5 \text{Uml}^{-1}$ ). The medium to shake flask volume ratio of 1:10 gave highest protease production ( $5.63 \times 10^5 \text{Uml}^{-1}$ ). The strain was cultivated at different temperatures; (37, 42, 50, 55, 60 and 65°C) and growth ( $\text{OD}_{610\text{nm}}$ ) was measured at different time intervals. The highest growth ( $\text{OD}_{610\text{nm}}$  1.788) and protease production ( $5.57 \times 10^5 \text{Uml}^{-1}$ ) were observed at 42°C at 84h respectively. Cultivation pH was varied from 6.0 to 8.0 and optimum pH for enzyme production was 6.75 ( $5.58 \times 10^5 \text{Uml}^{-1}$ ). To optimize the age of the slant used for inoculums preparation, slants containing different aged bacteria (18, 24, 30, 36, 42 and 48h) were used. There was no change in the enzyme production when different aged slants were used for inoculum preparation. Age of the inoculum was varied from 5 to 12h and highest enzyme production ( $5.51 \times 10^5 \text{Uml}^{-1}$ ) was obtained with 8h old inoculum. Size of the inoculum was from 10 to 30% and highest production ( $5.64 \times 10^5 \text{Uml}^{-1}$ ) was obtained with 20% (v/v) inoculum. Further studies are in progress to improve protease production from *Bacillus licheniformis* M27.