Improvement of Bacillus licheniformis M27 for Protease Production

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This study was aimed to improve the protease production by *Bacillus licheniformis* M27 (CFTRI, Mysore). Single colonies of the strain were transferred to nutrient-agar slants and grown at 37°C for 24h. The nutrient-agar medium contained (gl^{-1}) nutrient broth, 10.0; peptone, 10.0; sodium chloride; 5.0, and bacteriological agar, 17.5. The bacterial cells grown on the slants were transferred into activation medium and incubated in a shaker water bath at 42°C and 100 rpm for 18h. The activated strains were used as inocula and inoculated (20% v/v) to fermentation medium. Fermentation was carried out in shaker water bath at 42°C and 100rpm. Samples were taken at different time intervals and tested for protease activity measurement. The activity of protease was measured by estimating the amount of tyrosine released from casein. Both activation and fermentation media were same and contained (gl^{-1}) $(NH_4)_2SO_4$ 10.0; peptone, 4.0; glucose, 6.0; Na_2HPO_4 8.0; KH_2PO_4 4.0; $MgSO_4.7H_2O$, 0.5; and $CaCl_2.2H_2O$, 0.02. Concentration of glucose and nitrogen sources in the fermentation medium was optimized to improve the protease production. The $(NH_4)_2SO_4$ concentration in the medium was increased from 0 to $15gl^{-1}$ while all the other components of the fermentation medium was kept constant. The highest protease activity ($2.17x10^5$ Unit ml^{-1}) was produced in the fermentation medium containing $10gl^{-1}$ $(NH_4)_2SO_4$. Then to the fermentation medium containing $10gl^{-1}$ $(NH_4)_2SO_4$, different concentrations of peptone were added (0 6 gl^{-1}) and highest protease activity (2.30×10^5 Unit ml^{-1}) was obtained in the medium containing $2gl^{-1}$ peptone. The ratio between $(NH_4)_2SO_4$ and peptone concentration was varied (from 1:1 to 1:8) while keeping the total nitrogen concentration constant ($2.4gl^{-1}$). The highest protease activity (4.13×10^5 Unit ml^{-1}) was obtained in the medium which had a nitrogen ratio between $(NH_4)_2SO_4$: peptone as 1:4. Thus, the amounts of $(NH_4)_2SO_4$ and peptone were optimized as 2.26 and $14.22gl^{-1}$ respectively. To the medium containing optimized amount of $(NH_4)_2SO_4$ and peptone, glucose of different concentration varying from 3 to $12gl^{-1}$ was added and $7.5gl^{-1}$ glucose containing medium gave the highest protease activity (1.27 times higher than the control). While optimizing the medium, the strain improvement studies were also carried out. Initial cells in activation medium was $3.02x10^7$ ml^{-1} and 254Hz UV-irradiation was applied from 6cm of distance to (growth time in activation medium was 18h) bacterial cells. Viable cells were counted at different time intervals. The number of viable cells decreased suddenly to 19% in 10 min; and there after decreased very slowly. When the UV exposure times were 40, 60 and 80 min, the percentages of survival cells were 5, 3, and 2.5% respectively. Samples, which were UV-irradiated for 40, 60 and 80 min, were diluted serially and spread evenly on nutrient agar plates. Selected single colonies were activated and fermentation was carried out. Samples were taken at different time intervals and tested for protease activity. After 72h, protease activity increased by 1.43 times in a colony (from 60 min irradiated sample) and by 1.60 time in another one colony (from 80 min irradiated sample). These two strains were named as UV_A and UV_B respectively and they were kept for second cycle of UV-irradiation. In the second cycle, UV-irradiation was applied for 80min. From each of UV_A and UV_B 12 colonies were selected. The same procedure was carried out as in the first cycle of UVirradiation. The activity was increased by 1.06 times in a colony from UV_A and by 1.13 times in another one colony from obtained UV_B. These two strains were named as UV_{A1} and UV_{B1} and further studies are carried out to improve the strain.