Selection and improvement of a bacterial strain for protease production

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This study was aimed at selecting a bacterial strain and to improve the strain for protease production. Bacterial strains available in the laboratory, which were isolated from cow dung (CD) and boiled rice extract (BRE), *Bacillus licheniformis* M27 (CFTRI, Mysore) and *Bacillus licheniformis* (ATCC, 6346) were used in this studies. Single colonies of the selected bacteria were obtained by cultivating the organisms in nutrient-agar medium at 37°C for 24h. Single colonies 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 shaker water bath at 42°C and 100 rpm for 18h. 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. The fermentation medium was inoculated with the activated bacteria (20%, v/v) and incubated. Strains CD, BRE, *Bacillus licheniformis* M27 and *Bacillus licheniformis* (ATCC, 6346) reached lag phase at 10.0,14.0, 8.0 and 14.0h respectively. Highest optical density (OD 610nm) values were obtained for CD, BRE, *Bacillus licheniformis* M27 and *Bacillus licheniformis* (ATCC, 6346) were 0.987, 1.087, 1.086 and 1.166 respectively. The highest protease activity ($1.54x10^3$ Unit ml^{-1}) was obtained for *Bacillus licheniformis* M27 at 48h. CD, BRE and *Bacillus licheniformis* (ATCC, 6346) gave the highest activities of $1.64x10^3$, $5.2x10^2$, $7.5x10^2$ Unit ml^{-1} respectively at 72h. Therefore *Bacillus licheniformis* M27 is the most suitable strain for further studies because it gave the highest activity at short time of fermentation than other strains. The protease activity from the bacterial strain of CD, BRE, *Bacillus licheniformis* M27 and *Bacillus licheniformis* (ATCC, 6346) showed zero order kinetics for 17.5, 180, 25 and 20 min respectively. Therefore the incubation period for the protease obtained from the different bacteria was fixed as 10min. The activities of protease from all four strains were measured at different temperature from 30 to 90°C. The optimum temperature for the activities of all four proteases was 70°C. The proteases from *Bacillus licheniformis* M27 showed optimum activity ($5.9x10^4$ Unit ml^{-1}) at pH 9.0. Protease showed the K_m value with casein as $0.118gl^{-1}$ at pH 9.0 and 70°C. The enzyme incubated at 4°C and room temperature (32°C) retained 100% of the initial activity for 20 days at pH 6.2. The enzyme incubated at pH 6.2 and 70°C lost 90% of its original activity in 10min, while that at 60°C retained 70% of its initial activity at 1h. When the enzyme was incubated with $25gl^{-1}$ casein at pH 9.0, it retained 90% of its initial activity at 70 (2h) and 60°C (4h) respectively. Further studies are in progress to improve the strain and to optimize the fermentation medium and culture conditions to increase the production of protease enzyme.