

## Isolation and Selection of Thermo Stable Alkaline Protease Producing Bacterial Strains

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The objective of this study is to select the best alkaline protease producer from locally isolated bacterial strains. For this purpose 92 bacterial strains were isolated from dog (61Nos), beef (17Nos) and fish (14Nos) decaying soil. Single colonies of the isolated bacterial strains were cultivated in nutrient-agar medium at 40°C for 24h. The nutrient agar medium contained (gL<sup>-1</sup>) nutrient broth, 10.0; peptone, 10.0; sodium chloride, 5.0; and bacteriological agar, 17.5 at pH 7.0. The bacterial cells grown on nutrient-agar medium were transferred to the activation medium, incubated in shaker water bath at 40°C and 120 rpm for 18h. Then they were inoculated to the fermentation medium and incubated in a shaker water bath at 40°C and 120 rpm for 144h. Both the activation and fermentation media were same and contained (gL<sup>-1</sup>) glucose, 10.0; peptone, 5.0; yeast extract, 5.0; KH<sub>2</sub>PO<sub>4</sub>, 10.0; MgSO<sub>4</sub>.7H<sub>2</sub>O, 0.2; and Na<sub>2</sub>CO<sub>3</sub>, 10.0; at pH 9.5. Among the 92 bacterial strains, selected 36 strains produced the alkaline protease activity, above 4 UmL<sup>-1</sup> when the protease activity was measured at 70°C and pH 9.5. These 36 strains showed different morphological characters. Single colonies with different size [1mm (5), 2-4mm (17) and 5-7mm (14)], shape [round (19), irregular (4), tip-splitting (2), chiral morphotype (1) and filamentous (10)], elevation [flat (22) and low convex (14)], colour [pale (17), white (4) and yellow (15)] and margin [entire (16) and irregular (20)] were observed. Among the 36 alkaline protease producer's, five strains which gave alkaline protease activity in the range from 90 to 1760 UmL<sup>-1</sup> were selected and labelled as DS<sub>1</sub>, DS<sub>2</sub>, DS<sub>3</sub>, DS<sub>4</sub> and DS<sub>5</sub> respectively. The strains DS<sub>1</sub>, DS<sub>2</sub>, DS<sub>3</sub>, DS<sub>4</sub> and DS<sub>5</sub> showed maximum proteases production at 72, 80, 108, 66 and 96h respectively and gave the highest protease activities of 140.7 (72h), 1760 (80h), 1420 (108h), 92 (66h) and 1512 (96h) respectively. The half lives of the enzymes from the strains DS<sub>1</sub>, DS<sub>2</sub>, DS<sub>3</sub>, DS<sub>4</sub> and DS<sub>5</sub> were 26, 44, 41, 11 and 39 minutes at 70°C and pH 9.5 respectively. Among the 5 strains, the strain DS<sub>2</sub> produced the highest protease activity and the enzyme showed highest thermo stability. Strain DS<sub>2</sub> was isolated from dog decaying soil.

**Key words:** Protease, thermostable, isolation, strains and half life.