Production of α-amylase from locally isolated thermotolerant *Bacillus* strain

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The aim of this work is to produce a thermostable α- amylase from a bacterial strain. In this study a *Bacillus* strain isolated from rice broth *(Bacillus*, BR_1) was used for amylase production. The activation medium used for inoculum preparation contained (gl^{-1}) soluble starch, 2.0 and nutrient broth, 25.0. The activation medium (20ml) was inoculated with 2 loops full of the bacteria from stock culture and incubated at 45°C and pH 7.0 (100 rpm). At 16h, the temperature was increased to 50°C and incubated for 8h, (100 rpm). Inoculum (20%, v/v) was transferred into fermentation medium (pH 7.0) and incubated at 50°C. The fermentation medium contained (gl^{-1}), soluble starch, 2.0; $CaCl_2.2H_2O$, 0.005; $MgCl_2$ $6H_2O$, 0.005; $FeCl_3$, 0.005; K_2HPO_4, 2.5; KH_2PO_4, 10.0; peptone, 2.0; NaCl, 1.0; and $(NH_4)_2SO_4$, 2.0 The maximum enzyme activity of 20 Units ml^{-1} was obtained at 72h in the fermentation medium (1 Unit = μmole/minute). When the enzyme assay was done in presence of Ca^{++} (150 ppm), the phosphate present in the fermentation medium precipitated the calcium as calcium phosphate. The enzyme showed maximum activity at 85°C. The enzyme in the spent medium lost 95 and 18% of the original activity in presence and absence of calcium (150 ppm), at 30 min of incubation at 85°C. This was due to the precipitation of the enzyme in the fermentation medium in presence of calcium at 85°C. Hence in the next set of experiment different amounts of K_2HPO_4 and KH_2PO_4 (2.5 & 10.0, 1.25 & 5.0, 0.625 & 2.5 and 0.0 & 0.0gl^{-1}) were added to the fermentation medium, to find the minimum amount of the salts required for fermentation. The enzyme activity obtained was 20, 12, 3.3 and 2.4 Units ml^{-1} respectively at 72h. Hence it was decided not to decrease the salts. To the enzyme dialyzed against distilled water to remove the phosphate for 7h at 20°C Ca^{++} (150 ppm) was added, the enzyme retained 32 and 73% of its original activity respectively at 3h in the absence and presence of Ca^{++} at 85°C. To study the effect of temperature on the growth and fermentation character of BR_1, the organism was activated at 45°C and grown in the fermentation medium at 30, 42, 50 and 60°C. Maximum growth (OD_{600nm}, 1.27) was observed at 42°C while highest α-amylase activity produced was at 50°C (20.0Units ml^{-1}, 3rd day). The enzymes produced by BR_1 at 42°C and 50°C were taken and their activities at different temperatures were measured. Both enzyme samples showed maximum activities at 85°C. Therefore the growth temperature of the organism did not influence the optimum activity temperature of the enzyme.