INCREASE IN STABILITY OF XYLANASE FROM AN ALKALOPHILIC THERMOPHILIC BACILLUS (NCIM 59)

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Summary
The cellulase-free xylanase from an alkalophilic thermophilic Bacillus was stable at pH 7.0 to 10.0 at 50° for 3 days. At 60° the enzyme showed a decrease in stability with a half-life of 3 h. Addition of various additives had no effect on the enzyme stability at 60°. Glycine (0.5M) increased the enzyme half-life 6-fold at pH 7.0 to 9.0 and at 60 and 70°. Xylan could offer protection against thermoinactivation of the xylanase at pH 7.0 and 8.0 at 60° and only a marginal increase at pH 9.0 at 70° was observed.

Introduction
Cellulase-free xylanases have been the focus of recent attention due to their application potential in paper and pulp industries [Jurasek and Paice, 1986]. Xylanases stable and active at high temperature and alkaline conditions are suitable for biotechnological application. Enhancement of the half-life of the enzyme at elevated temperature would be desirable. An industrial disadvantage of the commercially used biocatalysts and enzyme complexes is their relatively low stability. One way to identify thermally stable enzymes is to exploit natural sources such as thermophilic organisms. They are known to produce enzymes with higher thermostability than those from their mesophilic counterparts [Nicolson et al., 1988]. The stability of the enzymes can also be increased by chemical modification and cross-linking, immobilization, treatment with additives polyols or osmolytes [Gupta, 1991] or by protein engineering [Wetzelet al., 1988]. Protein engineering requires sophisticated techniques and the knowledge of the gene sequence of the protein. Addition of low molecular weight additives to change the microenvironment of the enzyme solution is a practical way of stabilization. Earlier we reported the isolation of an alkalophilic thermophilic Bacillus NCIM 59 (AT Bacillus) that produces cellulase-free xylanase [Hinge et al., 1989]. The purification and properties of the xylanase from this organism has been documented [Dey et al., 1992]. Chemical modification studies indicate that the presence of tryptophan and carboxylic acid are essential for the activity of the enzyme [Chauthaiwale and Rao, 1993; 1994].
The xylanases from AT Bacillus are stable at 50°C which is the cultivation temperature of the organism. However, at higher temperature and alkaline pH the enzyme loses activity. The present paper reports the effect of addition of substrate and various additives on the stability of the xylanase.

Materials and Methods
Enzyme production: Xylanase from AT Bacillus [Hingeet al., 1989] was produced in a 50 ml medium containing wheat bran (5%) and yeast extract (1%), with 1% Na₂CO₃. The centrifuged culture broth was precipitated with 3 volumes of chilled ethanol as described by (Dey et al., 1992). The precipitate was dissolved in 10 ml of 50mM potassium phosphate buffer, pH 7.0 and was used for stability experiments.

Enzyme assay: Xylanase was assayed by mixing the enzyme with 0.5 ml of xylan solution (1%) in a final volume of 1 ml and incubating at 50°C for 30 min. The reducing sugars released were determined by dinitrosalicylic acid method (Miller, 1959). One unit (U) of xylanase activity was defined as the amount of enzyme that produced 1 μmole of xylose equivalent per min from xylan under the assay conditions.

Thermal and pH stability: The stability of the enzyme was measured by incubating 10 U in 0.1 ml of volume for different time intervals at desired pH and temperature. The effect of various compounds on the stability was determined by incubating the enzyme in presence of the additives. At the end of incubation the enzyme was cooled for 5 min and the residual activity was determined. The enzyme at 4°C in the absence of additive was assumed to have 100% activity.

Results and Discussion
The xylanase from AT Bacillus was stable for 3 days at 50°C. However, at higher temperature the enzyme showed decrease in half-life. The protective effect of different additives on the stability of the enzyme at 60°C was studied. Polyhydric alcohols such as glycerol, sorbitol and mannitol and sugars like glucose, sucrose, xylose at 10% concentration each did not modify the stability of the enzyme. Similarly, various salts such as CoCl₂, CaCl₂, KCl and NaCl at 10mM concentration each, did not have any effect. However, neutral amino acids glycine (0.5M) and β-alanine (0.5M) increased significantly the stability of the xylanase enzyme at 60°C. The effect of the amino acids on xylanase stability was investigated in detail.

The xylanase from AT Bacillus is stable at pH 7 and 8 at 60°C with half-lives of 3 and 2 h, respectively. However, at pH 9 and 10 the native enzyme lost 50% activity in 15 and