Effect of Selected Polyols And Salts on Stability of Xylanase Produced By *Bacillus pumilus*

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ABSTRACT

The objective of this study is to improve the stability of an alkaline xylanase with selected polyols and salts, which was produced by a locally isolated alkalo-thermophilic *Bacillus pumilus*. Xylanases with higher stability are used for the application in paper and pulp industry for bio bleaching. In the absences of additives at 55°C, the xylanase retained 38(±1.0)% of its initial activity at 30min and retained 45.6 (±0.76) and 20.6(±0.84)% of its initial activity at pH 8.0 and 9.0 respectively. Xylanase which contained polyols such as 10mM Poly Ethylene Glycol (PEG)-8000, 1M glycerol and 2M sorbitol the enzyme retained 24.0(±0.34), 19.0(±0.84) and 53.8(±0.57)% of its initial activity respectively at 60min. Addition of 10mM NaCl lost all of its activity while the enzyme retained 88.4(±0.18)% of its initial activity in the presence of 10mM$CaCl_2$. Sorbitol and $CaCl_2$ were the best additives among the polyols and salts respectively. $CaCl_2$ and sorbitol of different concentrations were studied and the half-lives of the xylanase in presences of 10mM $CaCl_2$ and 2M sorbitol were 302 and 63min respectively. When both 10mM $CaCl_2$ and 2M sorbitol were used together, the enzyme retained more (95%) of its initial activity at 60min than that in presence of 10mM $CaCl_2$ (88%) and 2M sorbitol (53%) individually. At 55°C half-life of the xylanase in presence of $CaCl_2$, sorbitol and $CaCl_2$ & sorbitol were 18, 47 and 552min respectively. The enzyme contained both $CaCl_2$ & sorbitol helped to retain 95, 88 and 18% of the initial activity at 55, 60 and 65°C respectively; while at 70°C it lost all of its activity at 120min. Xylanase from *B.pumilus* was stable at 60°C for 2h with both $CaCl_2$ & sorbitol.

Key words: Half-life, Polyols, Stability, Salts, Xylanase

1. INTRODUCTION

An increasing concern over environmental pollution has new challenges for the development of Biotechnological processes. Due to the environmental friendly nature, xylanases are used for their application in paper and pulp industry [1, 2, 3] for bio bleaching. Application of xylanase in this industry was effective in decreasing the amount of chlorinating agents [4]. Stability of xylanase at high temperatures that is in the range of 60-70°C is expected to increase its suitability for application in paper and pulp industry [5]. The stability of enzyme can also be increased by chemical modification, cross-linking, immobilization, treatment with additives and protein engineering [6]. Inclusion of additives to enzyme solution changes its microenvironment and provides a simple but practical means of increasing the stability of the enzyme [5, 7, 8]. In the present investigation, the effects of different additives such as polyols and some salts on the thermal stability of xylanase from *B.pumilus* were studied.

2. MATERIALS AND METHOD

Materials

Birchwood xylan was from Roth, Germany and peptone, yeast extract, PEG-8000, NaCl, $CaCl_2$, glycerol & sorbitol were from Sigma Chemical Company, USA.

Strain

In this study xylanase produced by locally isolated and identified *Bacillus pumilus* was used [9].

Culture Media and Production of xylanase

The Xylan Nutrient Agar plates and slants containing (gL^{-1}) nutrient agar, 28.0 and Birchwood xylan, 20.0 at pH 8.5 was used for the storage of the isolates and incubated at 40°C for 24 h.