Expression and characterization of Geobacillus stearothermophilus SR74 Recombinant α-Amylase in Pichia pastoris

ABSTRACT

Geobacillus stearothermophilus SR74 is a locally isolated thermophilic bacteria producing thermostable and thermoactive α-amylase. Increased production and commercialization of thermostable α-amylase strongly warrant the need of a suitable expression system. In this study, the gene encoding the thermostable α-amylase in G. stearothermophilus SR74 was amplified, sequenced, and subcloned into P. pastoris GS115 strain under the control of a methanol inducible promoter, alcohol oxidase (AOX). Methanol induced recombinant expression and secretion of the protein resulted in high levels of extracellular amylase production. YPTM medium supplemented with methanol (1% v/v) was the best medium and once optimized, the maximum recombinant α-amylase SR74 achieved in shake flask was 28.6 U mL−1 at 120 h after induction. The recombinant 59 kDa α-amylase SR74 was purified 1.9-fold using affinity chromatography with a product yield of 52.6% and a specific activity of 151.8 U mg−1. The optimum pH of α-amylase SR74 was 7.0 and the enzyme was stable between pH 6.0–8.0. The purified enzyme was thermostable and thermoactive, exhibiting maximum activity at 65°C with a half-life (t1/2) of 88 min at 60°C. In conclusion, thermostable α-amylase SR74 from G. stearothermophilus SR74 would be beneficial for industrial applications, especially in liquefying saccrification.

Keyword: Geobacillus stearothermophilus; Bacteria; Amylase; Strain