Characteristics of recombinant maltogenic amylase from Geobacillus sp. SK70

ABSTRACT

A thermostable maltogenic amylase producing-bacterium was isolated from a 70°C hot spring at Sungai Klah, Perak, Malaysia and was designated as Geobacillus sp. SK70 based on the 16S rRNA gene analysis. The gene encoding a thermostable maltogenic amylase was expressed in Escherichia coli using pET102 Directional TOPO expression vector, and it is the first ever report on using such expression vector. The highest enzyme activity was obtained after 12 h of post-induction time using 0.02 mM isopropyl β-D-thiogalactopyranoside (IPTG). The enzyme was purified to homogeneity with 8.2-fold and 41% recovery through a single-step using His-Trap HP affinity column chromatography. The optimum temperature and pH of the purified enzyme was at 55°C and pH 7.0, respectively, and showed broad pH stability ranging from pH 5.0 to 10.0. The activity of the purified enzyme was stable in the presence of 1 mM Ca2+;stimulated by 1 mM Mn2+and Zn2+, and 0.1 % (v/v) Tween-20; and inhibited by 1% (v/v) of 2-mercaptolethanol, EDTA and SDS. Thus the enzyme could be considered Ca2+-independent, which demonstrated characteristic unlike other reported maltogenic amylases, and offered good characteristics for industrial applications.

Keyword: Ca2+-independent; Geobacillus sp.; Maltogenic amylase; thermostable; Zn activated