Identification of a high catalytic efficiency beta-amylase from *Bacillus aryabhatai* GEL-09

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Abstract

β -amylase (EC 3.2.1.2), catalyzing the hydrolysis of the second α -1,4 glycosidic bond from non-reducing end of amylose or amylopectin, is a exo-type enzyme that plays a significant role in the production of maltose syrup. In this study, a novel β -amylase (amyB) was characterized from *Bacillus aryabhatai* GEL-09. It showed that the amyB encoding gene was composed of 1542 bp, which encodes a protein containing 514 amino acid residues. The amino acid sequence alignment revealed that the β -amylase from *Bacillus aryabhatai* shared the highest identity of 74% with that from *Bacillus cereus*. The amyB was over-expressed in *E. coli* BL21(DE3) and the recombinant enzyme was purified to homogeneity. The molecular weight of the recombinant β -amylase was estimated to be about 57.6 kDa by SDS-PAGE. The recombinant β -amylase has maximum activity at 50 °C, and pH 6.0, and it maintained 53, 72 and 94% relative activity at 30, 40 and 60 ° C, respectively. In the presence of Co²⁺, the activity of the recombinase was enhanced and was inhibited by EDTA, and other metal ions had little effect on the activation or inhibition of the enzyme. The kinetic analysis showed that when the soluble starch was used as the substrate, the michaelis constant (K_m) was 9.9 mg mL⁻¹, the catalytic constant (K_{cat}) was 116961.1 s⁻¹, and the K_{cat}/K_m was 11733.7 mL s⁻¹ mg⁻¹. The enzyme showed the highest specificity for soluble starch, followed by corn starch. Then the recombinant β -amylase was used to transform soluble starch (10%) into maltose, the yield of maltose reached 55.14% (w/w) after 24 h. The β -amylase identified in this study has potential applications in the starch sugar industry.

Results

Fig.1 Multiple sequence alignment of β -amylase protein sequences indicating highly conserved residues highlighted by red box. Accession numbers of the β -amylases are as follows: *Hordeum vulgare* subsp. spontaneum(P82993.1), *Hordeum vulgare* (P16098.1), *Zea mays* (P55005.1), *Medicago sativa* (O22585.1), *Vigna unguiculata* (O64407.1), *Glycine max* (P10538.3), *Ipomoea batatas* (P10537.4), *Arabidopsis thaliana* (O23553.3), *Oryza sativa Japonica Group* (Q10RZ1.1), *Bacillus aryabhatai* (O33580731.1), *Bacillus cereus* (P36924.2), *Paenibacillus polymyxa* (P21543.1), *Bacillus firmus* (P96513.1), *Bacillus circulans* (P06547.1), *Thermoanaerobacterium thermosulfurigenes* (P19584.1). The six conserved regions (I, II, III, IV, V, VI) are shown by underline.

Results

Fig. 2 Phylogenetic tree has been constructed by NJ method based on β -amylase protein sequences from different plants and microorganisms. The evolutionary distance and branch length have been shown in the figure.

Fig. 3 SDS-PAGE analysis of β -amylase. Lane M, protein size standard; lane1, Fermentation supernatant of *E. coli* BL21(DE3)/pET20b-amyBary; lane2, Supernatant after precipitation of ammonium sulfate; lane3, Purification on DEAE Ion exchange column

Fig. 4 The effect of temperature and pH on the activity of β -amylase. (a) The effect of pH on the activity of β -amylase. The reaction was carried out in different buffers of 0.2 M Na₂HPO₄-Citric acid buffer (pH 4.0-8.0) at 50 ° C. (b) The effect of pH on the stability of β -amylase. The pH stability was measured by incubating enzyme for 12 h in the range of pH 4.0-8.0 at 4 ° C. The enzyme activity of the pre-incubation was determined to be 100% under optimal conditions. (c) The effect of temperature on the activity of β -amylase. The reaction was carried out in a 0.2 M Na₂HPO₄-Citric acid buffer at pH 6.0 at different temperatures from 30 to 70 °C for 10 min. (d) The thermal stability test of the recombinant β -amylase was carried out at 50 ° C and pH 6.0, and the β -amylase hydrolase activity was measured periodically.

Results

Table 1 Purification steps of recombinant β -amylase.

Purification steps	Total protein (mg)	Total activity (U)	Specific activity (U·mg ⁻¹)	Purification (-fold)	Yield (%)
Crude enzyme	174.5	75629.1	433.4	1	100
Concentrate enzyme	41.3	30970.2	749.9	1.7	41
DEAE-column	0.8	3039.1	3798.9	8.8	14.2

Table 2 Effects of metal ions and other reagents on the activity of recombinant β -amylase

Modulator	Concentration	Relative activity(%)
Cations		
Cu ²⁺	1mM	93.91
Zn ²⁺	1mM	98.78
Ca ²⁺	1mM	88.42
Mn ²⁺	1mM	99.39
Co ²⁺	1mM	115.11
Mg ²⁺	1mM	95.00
Fe ²⁺	1mM	94.52
Cu ²⁺	5mM	38.61
Zn ²⁺	5mM	115.20
Ca ²⁺	5mM	84.65
Mn ²⁺	5mM	94.13
Co ²⁺	5mM	114.44
Mg ²⁺	5mM	85.56
Fe ²⁺	5mM	96.24
Chelating agent		
EDTA	1mM	75.02
EDTA	5mM	62.84

Table 3 The kinetic paraments of recombinant β -amylase

Kinetic paraments	Data
V_{max} (U·mg ⁻¹)	6660.0±577.1
K_{cat} (s ⁻¹)	116961.1±10134.9
K_m (mg·mL ⁻¹)	9.9±2.1
K_{cat}/K_m (mL·s ⁻¹ ·mg ⁻¹)	11733.7±1016.7

Conclusion

In this study, the β -amylase gene amyBary from *B. arybhatai* was cloned and solubly expressed in *E. coli*. Recombinant β -amylase contained six conserved regions that were typical of all β -amylase. HPLC analysis demonstrated that the yield of maltose was 55.14% after 24 h. These results demonstrated that the β -amylase identified in this study has potential applications in the starch sugar industry.

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